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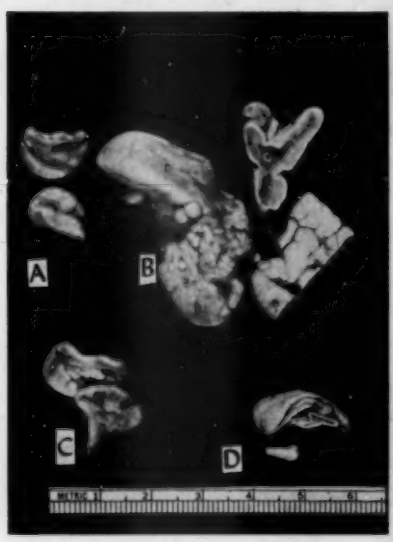
Complete Contents on First Page

From Nichols, p. 312



AMERICAN MEDICAL ASSOCIATION Publication

TABLE OF CONTENTS

VOLUME 62 OCTOBER 1956 NUMBER 4

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

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TO OUR SUBSCRIBERS AND CONTRIBUTORS

SUBSCRIBERS and contributors to this journal have undoubtedly noticed some deviation from our usual publishing quality in the July and August and September issues. In particular, authors have had to correct very bad proofs in less than adequate time for careful reading. Subscribers must have detected an unusual number of typographical errors which were not corrected from the galleys. A most disturbing fact was the late mailing of these issues. Many of our authors and readers were quick to realize that such a departure from our usual standards could be due only to abnormal conditions, and we feel that these conditions need to be explained.

On April 23, 1956, the American Medical Association concluded negotiations for a contract with the Baird-Ward Printing Company, Inc., Nashville, Tenn., to print the American Medical Association Specialty Journals. Under the terms of the contract, the Baird-Ward Printing Company, Inc., was to begin with the July issues.

A transfer of a printing operation requires that many details be considered and many adjustments be made. A contract as large as this involved expanding a work force with skilled operators, not always readily available; installing additional machinery and type equipment not obtainable on short notice, and scheduling nine journals so as to coordinate their production with other jobs in the plant. Editorially, it meant increased pressure upon editors to meet an advanced schedule, the development of a common interpretation of editing procedures between typesetter and manuscript editor, and the working out of communications between the Editorial Office, printing plant, authors, and readers.

All this was a Herculean task in view of the short period of time allowed for the transfer. Obviously, substandard results could not be avoided. While a contributor or a reader is justly concerned with his individual article or copy, it is hoped that the realization of the many ramifications of the transfer will soften criticism of the initial effort, performed under an entirely new set of production conditions.

Many of the early problems have been eliminated. Others are being solved daily. The efforts of the editorial and manufacturing staffs are directed toward an improvement over what our readers have been offered in the past. Publication dates will be improved, and plans are progressing to make our subject contribution more essential to the profession.

We trust that a better understanding of the circumstances will enlist your patient indulgence in our efforts to return to normal publishing conditions and to keep a promise of a better product.

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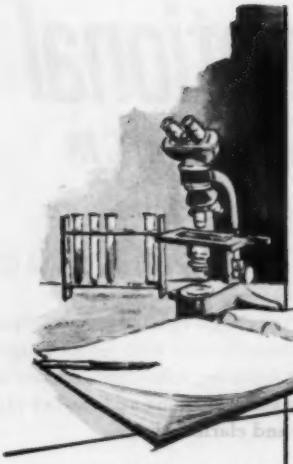

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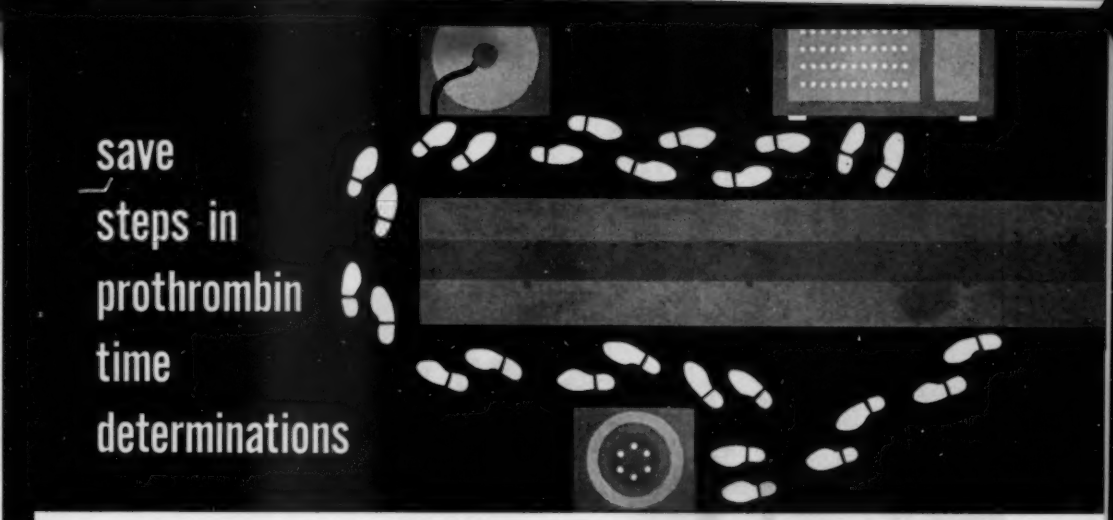

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Mitral Stenosis and Pulmonary Arteriosclerosis

Correlation of Pulmonary Arteriosclerosis, Right Ventricular Hypertrophy, and Thromboembolism in Autopsied Patients Who Died with Mitral Stenosis

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KYU TAIK LEE, M.D.
ERWIN R. RABIN, M.D.
and
R. M. O'NEAL, M.D., St. Louis

Arteriosclerosis of the small pulmonary arteries and pulmonary hypertension are common in patients with mitral stenosis,* and many observers have suggested that the pulmonary arteriosclerosis in such patients is caused by the hypertension.† However, evidence has been presented in recent studies of patients with mitral stenosis who have been subjected to mitral commissurotomies suggesting that the relationship between pulmonary hypertension and pulmonary arteriosclerosis has not been definitely established.[4]

Furthermore, evidence obtained from patients with congenital heart disease [5] and from animals subjected to pulmonary embolization ‡ indicates that in many situations thromboembolism can be a more important factor than hypertension in the development of pulmonary arteriosclerosis. We are unaware of any systematic studies of thromboembolism as a factor in the production of pulmonary arteriosclerosis in patients with mitral stenosis.

The purpose of this report is to present an analysis of the relationship of pulmonary arteriosclerosis, right ventricular hypertrophy, and pulmonary thromboembolism in 86 autopsied patients from Washington University who died with mitral stenosis. We have used right ventricular hypertrophy as presumptive evidence that pulmonary hypertension had been present during life.

Material and Methods

Autopsy records and microsections were examined from 86 patients with mitral stenosis as a principal anatomical diagnosis. These patients represent a consecutive series of all such patients over 20 years of age autopsied at Washington University during the period of 1938 to 1954 from whom suitable material was available for study. The paraffin blocks of Zenker-formalin-fixed tissue of lung from all patients were recut and stained with aldehyde-fuchsin-Van Gieson-iron hematoxylin (for elastic and connective tissue). In addition, other sections from most patients were prepared with hematoxylin and eosin, the periodic acid-Schiff technique, and Heidenhain's connective

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This study was supported by U. S. Grant H-1820 from the National Heart Institute, Institutes of Health, Public Health Service, Bethesda, Md.

From the Department of Pathology, Washington University School of Medicine. Assistant Professor in Pathology (Dr. Thomas). Life Insurance Medical Research Fund Fellow in Pathology (Dr. Lee). Senior medical student at Washington University when this study was done (Dr. Rabin). Instructor in Pathology (Dr. O'Neal).

* References 1, 2.

† References 1, 3.

‡ References 6-9.

tissue stain. All of these stains were used as described by Lillie,[10] except for minor modifications.

The microsections of lung were examined, and observations were recorded before any other information was obtained about the patients. Among the microscopic observations tabulated were the extent and severity of arteriosclerosis and the presence of thromboembolic phenomena.

The frequency and severity of arteriosclerotic lesions in the small pulmonary arteries were graded separately according to a system used previously by us in other studies.[8] Only the tissue stained with aldehyde-fuchsin-Van Gieson-iron hematoxylin was used for grading. A grade of 1 for frequency indicates that one to three lesions were encountered in each tissue section. A grade of 2 indicates four to six lesions, and a grade of 3, more than six lesions. The severity of the arteriosclerotic lesions was graded as follows: Grade 1 indicates a lesion that is less thick than the media; Grade 2 indicates a lesion as thick as the media, and Grade 3, one that is thicker than the media. In order to simplify charting of the results, grades for frequency and severity were combined and a single figure obtained as follows: If either frequency or severity was graded as 1, a combined grade of 1 ("slight") was assigned for combined frequency and severity. If both frequency and severity were graded as 2, a combined grade of 2 ("moderate") was assigned. If either frequency or severity was graded as 2 and the other graded as 3, or if both were graded as 3, a combined grade of 3 ("advanced") was assigned.

From the autopsy protocols prepared by house officers of the department of pathology at the time the autopsies were performed, we obtained the following types of information concerning each patient: age, sex, thickness of the right ventricular wall, degree of mitral valvular stenosis, and weight and gross appearance of the lungs.

For comparison, a "normal" (control) group of 50 patients with neither cardiac nor chronic pulmonary disease was selected by age from recently performed autopsies. An approximately equal distribution of patients was obtained from the 2d to the 10th decade of life.

Results

The results are summarized in Tables 1 through 3.

Forty-one per cent of the patients with mitral stenosis had "moderate" or "advanced" arteriosclerosis of their small muscular pulmonary arteries, whereas none of the patients in the "normal" (control) group had more than slight arteriosclerosis in their small pulmonary arteries (Table 1).

TABLE 1.—*Incidence of Arteriosclerosis in Small Pulmonary Arteries in Patients with Mitral Stenosis and in Controls*

Groups	Arteriosclerosis		
	Slight	Moderate	Advanced
Patients with mitral stenosis (86 patients)	51 (59%)	21 (24%)	14 (17%)
Controls (50 patients)	50 (100%)	0	0

TABLE 2.—*Correlation of Thickness of Right Ventricle with Presence of Arteriosclerosis in Small Pulmonary Arteries in Eighty-Six Patients with Mitral Stenosis*

Rt. Ventricle Wall, Mm.	Arteriosclerosis		
	Slight	Moderate	Advanced
6-13 (Ave. 8.7)	28 (60%)	13 (28%)	5 (12%)
2-5 (Ave. 4.1)	23 (58%)	8 (20%)	9 (22%)

TABLE 3.—*Correlation of Presence of Thromboembolic Phenomena in Lung with Presence of Arteriosclerosis in Small Pulmonary Arteries in Eighty-Six Patients with Mitral Stenosis*

Thromboembolism	Arteriosclerosis		
	Slight	Moderate	Advanced
Present (26 patients)	8 (30%)	9 (35%)	9 (35%)
Absent (60 patients)	43 (72%)	12 (20%)	5 (8%)

The patients with mitral stenosis were divided into two groups according to the thickness of their right ventricles, and the incidence of moderate or advanced arteriosclerosis was calculated for each group. In the group with right ventricular hypertrophy (whose right ventricles measured 6-13 mm. in thickness, average 8.7 mm.) the incidence of moderate or advanced pulmonary arteriosclerosis was 40%. The corresponding

incidence in the group without right ventricular hypertrophy was 42%. The right ventricles in the latter group measured 2-5 mm. and averaged 4.1 mm. in thickness, values which are similar to the range and average for the control group (range, 2-5 mm.; average 4.4 mm.). The difference between these percentages (40% and 42%) is not statistically significant. Thus, the incidence of moderate or advanced arteriosclerosis in the small pulmonary arteries in patients with mitral stenosis and right ventricular hypertrophy is similar to that in patients with mitral stenosis without right ventricular hypertrophy. In order to explore this matter further, we established a group consisting only of patients with mitral stenosis who had extreme right ventricular hypertrophy (with right ventricles 9 mm. or more in thickness; average 11.2 mm.). The incidence of moderate or advanced arteriosclerosis in this group (64%) was not significantly greater ($P=0.1$) than that in the patients with mitral stenosis whose right ventricles measured 2-5 mm. in thickness (43%). Arteriosclerosis was also found in the arterioles and in the large muscular arteries of patients in all groups, but in general it was less advanced in these vessels than in the small muscular pulmonary arteries. Sections of elastic arteries were available from too few patients to warrant analysis.

Evidence of obvious thromboembolism in the lungs was found in 32% of the patients with mitral stenosis (clearly recognizable organized or fresh thrombi or infarcts). Moderate or advanced pulmonary arteriosclerosis was present in 70% of the patients with thromboembolic phenomena, whereas only 28% of the patients without demonstrable thromboembolic phenomena had moderate or advanced pulmonary arteriosclerosis. This difference in incidences (between 70% and 28%) is highly significant ($P<0.01$).

Of the patients with mitral stenosis in this study 53% were men and 45% were women. The incidence of moderate or advanced pulmonary arteriosclerosis was not significantly different in the two sexes. However, it is interesting to note that among patients 20 to 50 years of age the incidence of Grade 2 or Grade 3 arterial lesions was twice as great among men as among women. Even though this difference is not great enough to be statistically significant ($P=0.1$), it does warrant further investigation in other series.

Comment

Numerous clinical and experimental studies have appeared from other laboratories indicating that thromboembolism is an important factor in the production of pulmonary arteriosclerosis in various circumstances.§ In previous studies of experimental animals and of patients with congenital heart disease, we have demonstrated in our own laboratory that thromboembolism can be a factor of primary importance in the production of arteriosclerosis in the small pulmonary arteries.‖ In the current study we have extended our observations by investigating the role of thromboembolism in the production of pulmonary arteriosclerosis in patients with mitral stenosis. In autopsied patients with mitral stenosis at Washington University the extent and severity of pulmonary arteriosclerosis in the small pulmonary arteries correlated closely with the presence of thromboembolic phenomena. Thus it appears from this study that thrombi are important in the production of pulmonary arteriosclerosis in patients with mitral stenosis.

Many observers have considered that pulmonary hypertension is an important factor in the production of pulmonary arteriosclerosis.¶ However, in patients with mitral stenosis autopsied at Washington University the extent and severity of pulmonary arteriosclerosis did not correlate with the presence of right ventricular hypertrophy. It seems reasonable to assume that a group

§ References 6, 7, 11.
‖ References 5, 8, 9.
¶ References 1, 3.

of patients with advanced right ventricular hypertrophy would have had higher pulmonary arterial pressures than a group of patients who did not have appreciable right ventricular hypertrophy. Thus it appears that pulmonary hypertension is of little or no importance in the production of arteriosclerosis in the small pulmonary arteries of patients with mitral stenosis.

In a recent study Goodale and his associates [4] examined biopsy specimens of lung from 50 patients with mitral stenosis who were subjected to mitral commissurotomies. The pulmonary arterial pressures of their patients had been determined by catherization studies. These investigators did not find a "close correlation" between the degree of pulmonary arteriosclerosis and the degree of pulmonary hypertension. Admittedly, a small biopsy specimen of the lung is not necessarily representative of the entire lung, and single, or even several, catheterization studies do not provide complete information regarding the degree and duration of pulmonary hypertension. Nevertheless, previously held views on the relationship between pulmonary arteriosclerosis and pulmonary hypertension led us to expect a close correlation between these two; but such a correlation was not demonstrated by either Goodale and his associates or by us.

In a recent review of the physiologic changes in mitral stenosis, Dexter [2] pointed out that it has been clearly established that mitral commissurotomy with relief of mitral stenosis is followed by the return of the pulmonary vascular resistance to normal. The almost uniformly favorable results from mitral commissurotomy have been surprising, since it has been established by many investigators, including ourselves, that obstructive lesions in the pulmonary vascular system can produce pulmonary hypertension by themselves.# Although our study had as its primary purpose to investigate the roles of thromboembolism and pulmonary hypertension in the production of pulmonary arteriosclerosis in patients with mitral stenosis, it provides a possible explanation for the favorable results obtained by mitral commissurotomy. Since the extent and severity of pulmonary arteriosclerosis did not correlate with the presence of right ventricular hypertrophy, our results suggest that the degree of obstruction produced by pulmonary arteriosclerosis in most patients with mitral stenosis is insufficient to account for a significant portion of the increased pulmonary vascular resistance in these patients. In other studies, we have been unable to demonstrate generalized structural changes in alveolar walls * or medial hypertrophy in small muscular pulmonary arteries.[14] Thus, it appears that the increased pulmonary vascular resistance in patients with mitral stenosis may be largely a result of physiologic alteration, rather than anatomical changes, and it is not surprising that after mitral commissurotomy pulmonary arterial resistance usually reverts to normal.

References 12, 13.

Summary

We have presented an analysis of the relationship of pulmonary arteriosclerosis, right ventricular hypertrophy, and thromboembolism demonstrated at autopsy in 86 patients with mitral stenosis. Right ventricular hypertrophy has been used as presumptive evidence that pulmonary hypertension had been present during life.

Approximately 40% of the patients who died with mitral stenosis had "moderate" or "advanced" arteriosclerosis of the small pulmonary arteries, whereas none of the patients in a control group had more than "slight" pulmonary arteriosclerosis.

There was no significant correlation between the presence of moderate or advanced pulmonary arteriosclerosis and the presence of right ventricular hypertrophy. This finding suggests that pulmonary hypertension is not an important factor in the production of arteriosclerosis of the small pulmonary arteries in patients with mitral stenosis.

* O'Neal, R. M.; Thomas, W. A.; Lee, K. T., and Rabin, E. R.: Alveolar Walls in Mitral Stenosis, to be published.

Correlation between the presence of moderate or advanced pulmonary arteriosclerosis of the small pulmonary arteries and the presence of thromboembolic phenomena in the lungs was highly significant ($P<0.01$), suggesting that the latter is an important factor in the pathogenesis of the former.

Although this study was concerned primarily with factors associated with pulmonary arteriosclerosis, it also has physiologic implications that may be useful in explaining the benefit derived from mitral commissurotomy. The absence of a significant correlation between the presence of moderate or advanced pulmonary arteriosclerosis and right ventricular hypertrophy suggests that the degree of pulmonary arteriosclerosis found in most patients with mitral stenosis is in itself insufficient to cause a significant increase in the pulmonary vascular resistance.

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Cancerogenic Effects of Ca^{45} and Sr^{89} on Bones of CF_1 Mice

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GLORIA E. ZANDER, M.A.
and
JOSEPH F. KUZMA, M.D., Milwaukee

Introduction

The present investigation was designed to study the long-term cancerogenic effects of Ca^{45} and Sr^{89} on the bones of CF_1 mice. Investigators at the Argonne Laboratories have reported that bone tumors can be induced in mice by single and monthly intraperitoneal injections of Sr^{89},* and also by single injection of Sr^{89} in dogs,† rabbits,‡ and rats.§ Studies in our own laboratory [9] have shown that both Sr^{89} and Ca^{45} produce bone tumors in Sprague-Dawley rats when given in single or monthly intraperitoneal injections.

Brues and associates,[2] at the Argonne Laboratories, reported bone tumors in CF_1 mice following single and monthly injections of Sr^{89} in doses ranging from 0.1 to 5 μc per gram of body weight. One of our present experiments was designed to study the effects of small weekly doses of Sr^{89} when given to mice over a long period of time. At the time the present investigation was begun, there were no published data on the cancerogenic action of Ca^{45} in mice. Two experiments were set up in this laboratory in order to study the long-term effects of Ca^{45} when it is injected intraperitoneally into mice as a single large dose and in small weekly doses. Recently Finkel and Scribner,[10] at the Argonne Laboratories, have reported bone tumors in mice following single intravenous injections of Ca^{45} and Sr^{90}.

Materials and Methods

Strain of Mice.—Virgin females of the CF_1 strain were obtained from Carworth Farms, New City, N. Y. These mice were chosen because they were readily available and because the supplier reported that to his knowledge no spontaneous bone tumors had ever been observed in mice of this strain during the period of their artificially limited life span as stock animals. It was not known, however, whether spontaneous bone tumors occurred in mice allowed to live their entire natural life span.‖ The mice were approximately 2 months old when the experiments were begun.

Ca^{45} and Sr^{89}.—The Oak Ridge National Laboratory supplied Ca^{45} and Sr^{89} in solutions of hydrochloric acid which were neutralized with sodium hydroxide. All injections were intraperitoneal.

Ca^{45} has a half-life of 163 days¶ and a maximum beta energy of 0.254 mev. Sr^{89} has a half-life of 53 days and a maximum beta energy of 1.463 mev. Shipments of Sr^{89} contained at least 10% Sr^{90}, which has a half-life of 25 years and a maximum beta energy of 0.61 mev. Sr^{90} decays to Y^{90}. The latter decays, with a half-life of 2.54 days and a maximum beta energy of 2.18 mev, to stable zirconium.

Submitted for publication June 4, 1956.

Present address: The University of Miami School of Medicine (Dr. Anderson).

From the Department of Pathology, Marquette University School of Medicine, Milwaukee.

This paper is based on work performed under contract No. AT (11-1)-105 with the U. S. Atomic Energy Commission, and is the completion of Document AECU 2624.

* References 2-6.

† References 7 and 8.

‡ References 2 and 3.

§ References 2 and 3.

‖ Finkel and associates [11] have since reported that spontaneous bone tumors (osteogenic sarcomas) appeared in 1% of the total population of their CF_1 mice (320 mice).

¶ When these experiments were begun, the half-life of Ca^{45} was reported as 180 days in the standard radioisotope handbooks. Consequently, all corrections for decay were based on the latter figure.

Plan of Experiments

Experiment A.—Experiment A was designed to study the effects of single large doses of Ca^{45}. The mice were weighed and injected according to body weight, as outlined in Table 1. The mice

TABLE 1.—*Plan of Experiment A on CF_1 Mice with Ca^{45}*

Group	Single Intraperitoneal Injection of Ca^{45}, μc per Gram Body Weight	Average Total Dose, μc	No. of mice
1	5.0	125.0	30
2	3.5	87.5	28

of Group 1 were injected with 5 μc of Ca^{45} per gram, and the mice of Group 2 were injected with 3.5 μc per gram. The average mouse body weight was 25 gm., so that the average total dose injected was 125 μc in Group 1 and 87.5 μc in Group 2. Group 3 consisted of 10 mice, which were maintained as controls. All the mice were weighed one month after injection and every six months thereafter.

TABLE 2.—*Plan of Experiment B on CF_1 Mice with Ca^{45}*

Group	Weekly Dose of Ca^{45}, μc (About One Year's Duration)	Estimated Total Dose Injected per Gram of Body Weight, μc	No. of Mice
1	2.10	3.5	20
2	1.50	2.5	20
3	0.60	1.0	20
4	0.30	0.5	20
5	0.06	0.1	20

Experiment B.—The object of Experiment B (Table 2) was to determine the effects of small weekly doses of Ca^{45}. The weekly injections extended over a period of approximately one year, so that two groups receiving a similar total dose per gram could be compared, i. e., Group 1, Experiment B, and Group 2, Experiment A. One hundred mice were divided into five groups of twenty each according to dosage, which ranged from 0.06 to 2.1 μc per week. Twenty mice were maintained as controls. In this experiment, the mice were weighed at the beginning of the injection period, at 4 months of age, and at 8 months of age.

Experiment C.—A long-term experiment with Sr^{90} was set up similarly to Experiment B, with Ca^{45}. It was planned to inject small weekly doses of Sr^{90} for about a year. For this purpose, 100 mice were divided into 5 groups of 20 each according to dosage, which ranged from 0.06 to 2.1 μc per week (Table 3). Another 20 mice were main-

TABLE 3.—*Plan of Experiment C on CF_1 Mice with Sr^{90}*

Group	Weekly Dose of Sr^{90}, μc (About One Year's Duration)	No. of Mice
1	2.10	20
2	1.50	20
3	0.60	20
4	0.30	20
5	0.06	20

tained as controls, bringing the total number of controls for Experiments A, B, and C to 50 mice. The experimental and control mice of Experiment C were weighed at the beginning of the injection period, at 4 months of age, and at 8 months of age.

Detection of Bone Tumors.—The experimental mice and controls were x-rayed once a month until death in order to detect bone tumors. Approximately one-third of the mice in each experimental group and all mice which had bone lesions of any kind were carefully autopsied. Histologic sections of these mice were prepared and examined.

Autoradiography of Bone Tumors.—Histologic sections were prepared of all bone tumors, and whenever the tumors were large enough to permit hemisection, half was used for the preparation of autoradiograms.# This half was fixed in an alkaline solution of 3 parts 95% alcohol and 1 part 40% neutralized formaldehyde. According to Bélanger,[13] this is a good fixative for mineral salts of bone and teeth.

After fixation the bone tumors were dehydrated and embedded in Ward's Bioplastic. Slices were made of the blocks by cutting them with a jig saw.

The surface radiation of these blocks was measured by counting them with a D-34 tube. The sensitivity of the emulsion and the surface concentration of the radioisotope being known, the exposure time was calculated. In some cases the tumors did not contain enough radioactivity to obtain an autoradiogram in a reasonable exposure time.

X-ray film was placed on the cut surface of the block and held in position by clamps. In most cases No-Screen x-ray film (Ansco) was used. After exposure the film was developed in Kodak

According to Boyd,[12] the term "autoradiogram" is preferable to "autoradiograph" in designating the result of the technique in which a photographic emulsion is placed in contact with a radioactive specimen.

x-ray developer (at 20 C) for three minutes and fixed for 10 minutes in Kodak acid fixer.

Results

Gross Observations.—EXPERIMENT A: Table 4 summarizes the data regarding incidence of bone tumors, latent periods, and survival after injection of single large doses of Ca^{45}. The anatomic location of the bone tumors is given in Table 5. Of the 30 mice which were injected with 0.5 μc of Ca^{45} per gram of body weight, 6 developed bone tumors, in an average latent period of 11.6 months. In the group given 3.5 μc per gram, only two mice developed bone tumors, in an average latent period of 14.8 months. It should be noted that an unusually large number of early deaths occurred in the latter group. Lung infections accounted for many of these deaths. None of the mice in Experiment A had multiple bone tumors. No significant differences in weight were noticed between the experimental mice and the controls at any time.

EXPERIMENT B: Small weekly doses of Ca^{45} were injected over a period of about a year. Table 6 shows the total dose of Ca^{45} which was given. The total dose injected per gram of body weight was estimated on the basis of an average weight of 30 gm. for an adult mouse.

TABLE 4.—*Experiment A on CF_1 Mice with Ca^{45}: Survival and Development of Bone Tumors*

Group	Single Intraperitoneal Injection of Ca^{45}, μc per Gram of Body Weight	No. of Mice	No. of Mice Which Developed Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Injection	Average Latent Period, Mo.	Survival		Incidence of Tumors in Those Mice Which Survived Average Latent Period*
						Bone Tumor Groups	Tumor-Free Groups	
						No. Surviving Average Latent Period or Which Died Sooner with Bone Tumors	Average Survival (in Mo.) Beyond Beginning of Experiment	
1	5.0	30	6	7.5	11.6	10	--	60.0%
2	3.5	28	2	12.5	14.8	4	--	50.0

* Those mice with bone tumors which did not survive the average latent period are included.

TABLE 5.—*Distribution of Bone Tumors in CF_1 Mice*

	Experiment A: Intraperitoneal Injections of Ca^{45} in Single Large Doses						
Original site of bone tumors	Left Foreleg	Right Foreleg	Left Hindleg	Right Hindleg	Skull	Spine	Pelvis
No.	0	0	0	0	2	4	2
	Experiment B: Intraperitoneal Injections of Ca^{45} in Small Weekly Doses						
Original site of bone tumors	Left Foreleg	Right Foreleg	Left Hindleg	Right Hindleg	Skull	Spine	Pelvis
No.	0	0	2	2	0	1	6
	Experiment C: Intraperitoneal Injections of Sr^{90} in Small Weekly Doses						
Original site of bone tumors	Left Foreleg	Right Foreleg	Left Hindleg	Right Hindleg	Skull	Spine	Pelvis
No.	1	0	2	1	0	0	0

TABLE 6.—*Experiment B on CF_1 Mice with Ca^{45}: Survival and Development of Bone Tumors*

Group	Weekly Dose of Ca^{45}, μc (About One Year's Duration)	Total Dose Injected in One Year, μc	Estimated Total Dose Injected per Gram of Body Weight, μc	No. of Mice	No. of Mice Which Developed Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Initial Injection	Average Latent Period, Mo.	Survival		Incidence of Tumors in Those Mice Which Survived Average Latent Period
								Bone Tumor Groups	Tumor-Free Groups	
								No. Surviving Average Latent Period or Which Died Sooner With Bone Tumors	Average Survival (Mo.) Beyond Beginning of Experiment	
1	2.10	105.70	3.5	20	6	11.5	14.8	14	----	42.9%
2	1.50	75.50	2.5	20	1	14.5	----	9	----	11.1
3	0.60	30.20	1.0	20	2	18.5	19.0	8	----	25.0*
4	0.30	14.90	0.5	20	0	----	----	----	5.6†	0
5	0.06	3.02	0.1	20	0	----	----	----	15.9	0

* It will be noted that the average latent period in this group was 19.0 months, in comparison with the average latent period of 14.8 and 14.5 months, respectively, in Groups 1 and 2. Twelve mice of Group 3 survived beyond 14.8 months. Based on this figure, the incidence of tumors in Group 3 would be 16.7%.

† Most of these mice died of Salmonella infection about four months after the beginning of the experiment. Only one mouse survived beyond eight months.

TABLE 7.—*Experiment C on CF_1 Mice with SR^{90}: Survival and Development of Bone Tumors*

Group	Weekly Dose of Sr^{89}, ^{90}, μc*	Total Dose Injected in 6.5 Mo., i.e., from First to Last Injection†	Estimated Total Dose Injected per Gram of Body Weight, μc	No. of Mice	No. of Mice Which Developed Bone Tumors	Minimum Latent Period (Mo.) of First Tumor After Initial Injection	Average Latent Period, Mo.	Survival		Incidence of Tumors in Those Mice Which Survived Average Latent Period
								Bone Tumor Groups	Tumor-Free Groups	
								No. Surviving Average Latent Period or Which Died Sooner with Bone Tumors	Average Survival (Mo.) Beyond Beginning of Experiment	
1	2.10	44.10	1.50	20	2	15.5	16.3	7	----	28.6%
2	1.50	30.50	1.00	20	1	13.5	----	9	----	11.1
3	0.60	12.20	0.40	20	0	----	----	----	15.9	0
4	0.30	6.10	0.20	20	0	----	----	----	14.2‡	0
5	0.06	1.22	0.04	20	0	---	----	----	5.7§	0

* Injections interrupted; see text.
† See text.
‡ Five mice of this group died of Salmonella infection about four months after the beginning of the experiment. The average survival for the remaining 15 mice of this group was 17.8 months.
§ Most of these mice died of Salmonella infection within six months after the experiment was started. Two mice survived 18 months.

The highest incidence of bone tumors occurred in those mice which were given the largest weekly dose, i. e., 2.1 μc (Table 6). No bone tumors appeared in those mice which received doses of 0.3 μc or 0.6 μc per week. Multiple bone tumors appeared in only one mouse. This mouse was given 2.1 μc per week. No consistent differences in weight were noted between the mice given weekly doses of Ca^{45} and the controls. It is interesting to note from Table 5 that in Experiments A and B, with Ca^{45}, bone tumors originated most frequently from the spine and pelvis (five tumors from the spine and eight from the pelvis).

EXPERIMENT C: Difficulties in procuring a regular supply of Sr^{89} necessitated interruption of the weekly injections, and finally the injections had to be abandoned. The last injection was given six and one-half months after the start of the experiment. Table 7 shows the total dose given to mice of Experiment C and the estimated dose injected per gram of body weight. The latter is based on an average weight of 30 gm. Two mice developed bone tumors in the group which received the highest weekly dose, i. e., 2.1 μc (Table 7). One of these mice had two bone tumors. Only one mouse developed a bone tumor in the next highest dose group (1.5 μc per week). Smaller doses produced no bone tumors. All the bone tumors in this experiment appeared in the limbs (Table 5). There were no significant differences in weight at any time between the experimental mice and the controls.

CONTROLS.—Two mice of the control group of 50 developed bone tumors, one at 16.5 months of age and another at 20 months of age. The average age of the control mice at the time of death was 18.7 months. These tumors measured less than 3 mm. in diameter at the time of death and were mature bone formations, called osteoma. Finkel and associates[11] have reported bone tumors (osteogenic sarcoma) in 1% of their control female mice of the CF_1 strain. The osteomas in the control animals of the present experiments are considered as spontaneous neoplasms, for the following reasons:

1. These bone tumors grew very slowly, if at all, from the time that they were first detected by x-ray until the death of the animal, whereas in the treated animals tumors appeared suddenly, grew rapidly, and usually measured more than 1 cm. in diameter at the time of death. No malignant bone tumor developed in the controls, and, although five of the treated animals had benign tumors, two of these also had malignant bone tumors which infiltrated the surrounding soft tissues.

2. No bone tumors appeared in the mice which received the smallest doses of radioactive calcium or strontium, indicating that a tumor developing in the bone of an untreated CF_1 mouse is a chance occurrence.

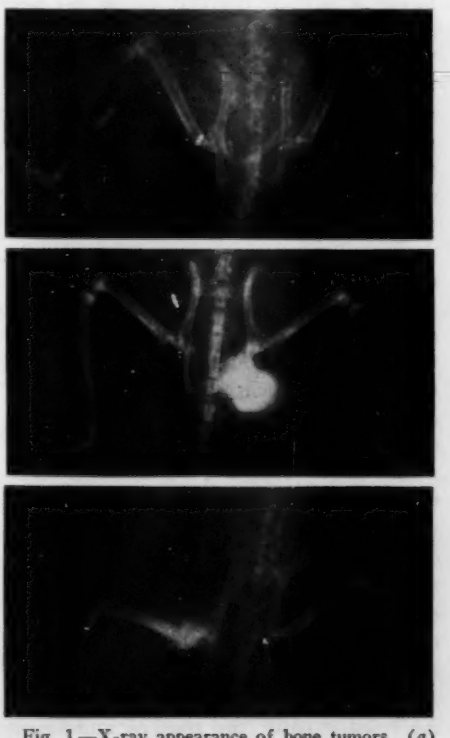

Fig. 1.—X-ray appearance of bone tumors. (*a*) Benign bone tumor (osteoma); (*b*) differentiated osteogenic sarcoma; (*c*) undifferentiated osteogenic sarcoma.

Pathology of Tumors

X-Ray Findings (Fig. 1*A*, *B*, and *C*).—Both the control and the treated animals were periodically examined by x-ray. The x-ray appearance of any tumor depends on the degree of bone differentiation, namely, the formation of trabecular or dense bone which is calcified, as determined histologically. The benign tumors (osteoma) appearing in the control and treated groups were uniformly dense, sharply outlined, and radiopaque. The adjacent soft tissues were displaced without fixation, infiltration, or development of soft-tissue mass adjacent to the bone lesion. Of the tumors which were malignant (osteogenic sarcomas), some were well differentiated and others were anaplastic. The better-differentiated malignancies also appeared quite dense but were marked by multiple spots which were radiolucent in various degrees, giving the total mass a sponge-ball appearance. Usually the borders were irregular. The least-differentiated tumors were also the least radiopaque and produced an ill-defined, fuzzy, infiltrating mass, accompanied by marked soft-tissue swelling surrounding the portions, which were radiopaque to some extent. In fact, soft-tissue masses were much larger in the anaplastic tumor group than in the others. The Ca^{45} group of animals usually showed tumors of increasing density, with an obliteration of the normal bone outline, whereas those produced by Sr^{89} showed a more irregular density and multiple zones of rarefaction within the bone at the point of origin.

Histology (Fig. 2*A*, *B*, and *C*).—The tumors occurring spontaneously in the control mice were of mature, compact, fully differentiated cortical type of bone. Much of this was developed as parallel layers of new-bone formation added to the cortex by the periosteum. The benign tumors occurring in the treated group were larger masses but of similar histology, although the point opposite the zone of attachment usually had more irregular orientation of its canals and laminations, without being parallel to the surface of the underlying bone.

The malignant tumors occurring in the animals given radioactive isotopes showed a considerable range of differentiation. Benign tumors (osteoma) developed in five of the animals, one of these having two separate osteomas. Two of the five animals with benign tumors also had a malignant bone tumor, while another one had a zone of malignant tumor formation within an "osteoma." The malignant tumors had a predominant pattern at some level of differentiation, but practically all of the tumors had a mixture of various degrees of differentiation. The stages of differentiation observed might be listed as follows:

1. Vascular tumor of pleomorphic cellular pattern with numerous giant cells and mitoses
2. Cellular "fibrous"-appearing tumors
3. Cellular fibrous tumor with tiny foci of intercellular osteoid and/or calcification
4. Cellular fibrous tumors with development of good osteoid calcification and presence of hyaline cartilage
5. Trabecular osteoid and bone formation of mature type
6. Any combination of these patterns

There was no consistent pattern in either the Ca^{45}- or the Sr^{89}-induced tumors.

Other Histologic Findings.—Three mice with bone tumors showed metastases in the soft tissues. Two had metastases in the lungs, and the other had a liver metastasis.

Ca^{45} and Sr^{89}, in the doses used in these experiments, produced no significant detectable pathologic changes in the soft tissues. Some minor degree of marrow atrophy and fibrosis occurred in three mice which were injected with Ca^{45} (Experiments A and B) and in three mice which were injected with Sr^{89} (Experiment C).

Two mice from each experimental group in Experiment A were killed eight weeks after a single injection of Ca^{45}. No significant histologic or gross changes were evident at that time.

Autoradiographic Findings (Fig. 3).—Autoradiograms were prepared of 2 bone tumors from Sr^{89}-injected mice and of 11 bone tumors which appeared in Ca^{45}-in-

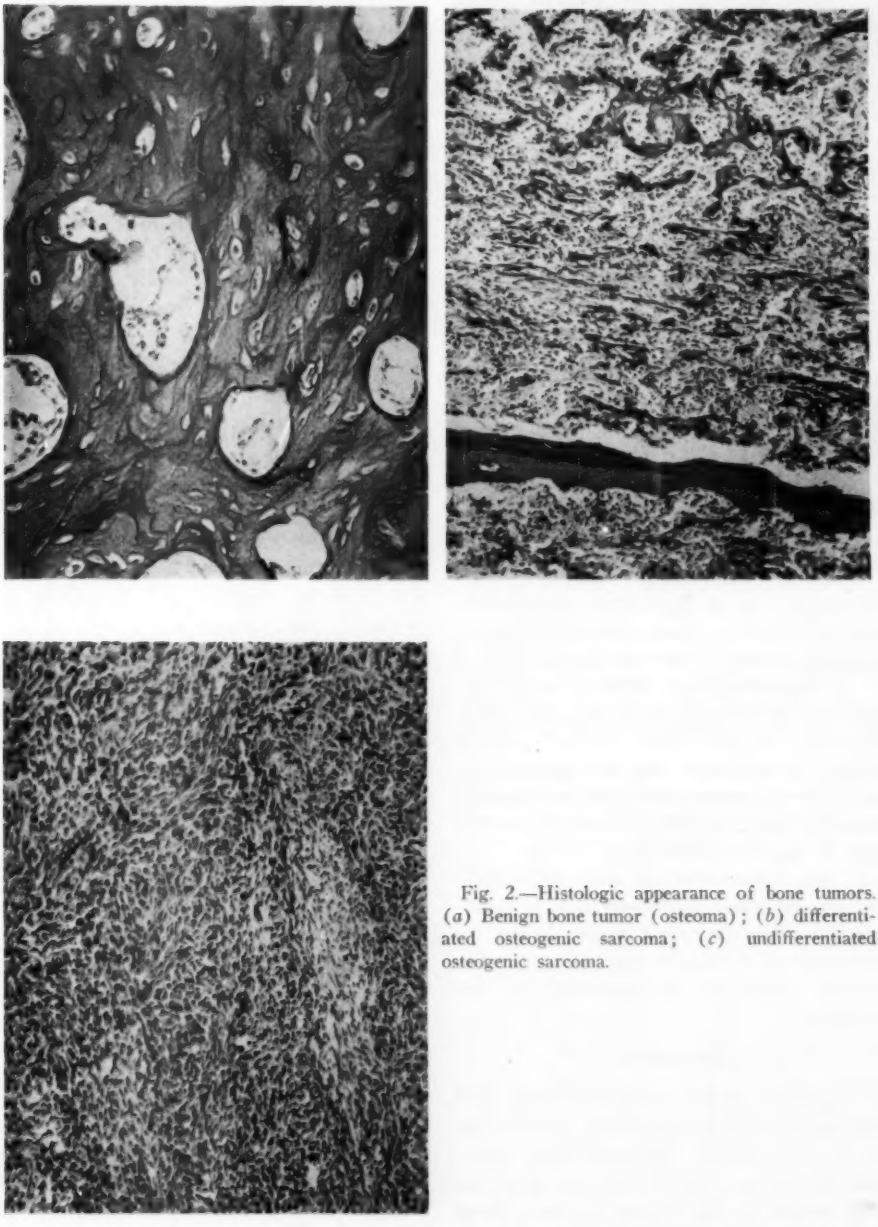

Fig. 2.—Histologic appearance of bone tumors. (*a*) Benign bone tumor (osteoma); (*b*) differentiated osteogenic sarcoma; (*c*) undifferentiated osteogenic sarcoma.

jected mice (5 from Experiment A and 6 from Experiment B). The following observations were made:

1. Sr^{89}, because of its high maximum energy of 1.463 mev, gave more diffuse autoradiograms than Ca^{45}. However, the localization of Sr^{89} or Ca^{45} did not differ significantly in the respective tumors.

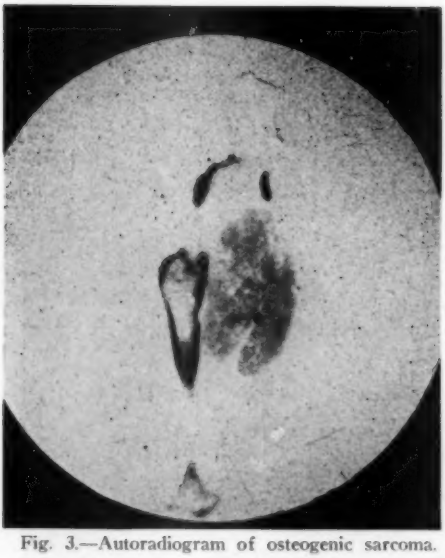

Fig. 3.—Autoradiogram of osteogenic sarcoma.

2. Radioactivity was present in the bone tumors to a much less extent than in the bone of origin. In some cases the bone of origin at the tumor site was completely lost in the autoradiogram, while in others the outline of the bone of origin was still intact. The latter was true of the two Sr^{89}-induced tumors from which autoradiograms were made. Both these tumors grew very rapidly from the time that they were first detected until the death of the animal.

3. Some tumors which were well calcified showed a heavy concentration of radioactivity within the tumor tissue. When the outer part of the tumor was soft, no radioactivity could be demonstrated in the periphery.

Comment

Long-term studies with Ca^{45} and Sr^{89} were carried out on adult virgin female mice of the CF_1 strain. Intraperitoneal injections of 3.5 or 5 μc of Ca^{45} per gram of body weight produced bone tumors. Bone tumors were obtained also in those mice which received 0.6 to 2.1 μc of Ca^{45} per week for approximately one year. Smaller doses produced no bone tumors. With a single large injection of Ca^{45} the total radioactivity within the bones gradually decreases with the progress of time. On the other hand, small weekly doses over a long period of time are associated with a rising level of radioactivity with the progress of time. However, it takes a much longer period to accumulate the "threshold dose" in bone which is needed for tumor development. If one adds to this the latent period of bone tumor formation, then the time at which a bone tumor appears will be much later in the life of the animal. In fact, the latent period may be projected beyond the normal life span; this may explain the failure of tumor production with the smaller doses (Table 6, Experiment B, Groups 4 and 5).

In the same strain of mice, bone tumors were also obtained after injection of Sr^{89} in weekly doses of 1.5 and 2.1 μc. The injections were concluded after six and one-half months because of difficulties in procuring the isotope.

Tumors in the Ca^{45}-injected animals showed a predominant distribution in the spine and pelvis, whereas the Sr^{89} animals of this experiment developed tumors only in the limbs. There is no explanation for this variation in distribution; however, it closely parallels that observed in Ca^{45} and Sr^{89} bone tumors produced in the rat.[9]

Two untreated (control) mice developed benign tumors of bone (osteoma), but none developed a malignant bone tumor. Benign tumors also developed in the Ca^{45}- or Sr^{89}-treated mice (5 of the 23 tumors were osteomas; however, there was also an associated malignant tumor in 3 of these 5).

Summary

Osteogenic sarcoma may be induced in virgin female CF_1 mice by Ca^{45} given intraperitoneally as a single injection or as multiple weekly injections.

Osteogenic sarcoma may be induced in virgin female CF_1 mice by Sr^{89} given intraperitoneally in multiple weekly injections. Tumors induced by Sr^{89} are found chiefly

in the limbs. Tumors induced by Ca^{45} are found chiefly in the spine and pelvis.

No malignant bone tumor developed in the control mice.

Of the 23 tumors in mice given Ca^{45} or Sr^{89}, 5 were osteomas.

Two control mice developed osteomas.

Benign tumors of bone are uniformly radiopaque and are sharp in outline.

Malignant tumors of bone are irregularly radiopaque and less well defined than are benign tumors.

Mr. Frank Karioris, of the Department of Physics at Marquette University, gave technical assistance.

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Tissue Reactions to Autologous and Homologous Musculofascial Transplants

Modifications by Thermal Treatment Before Implantation

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Introduction

Previous studies of normal autologous and homologous musculofascial transplants in rabbits have shown that they undergo similar basic patterns of degeneration and organization by vascularized granulation tissues of the recipient.[1] The pattern of organization of the homologous transplants, however, was characterized by restricted fibroblastic activity, impaired collagen deposition, and conspicuous lymphocytic infiltration, with varying degrees of angiitis involving predominantly the subfascial zones of the transplants. These features permitted accurate histological distinction between normal autologous and homologous musculofascial transplants, in spite of the fact that there was progressive deterioration of both types of grafts.

It seemed reasonable to suppose that factors responsible for development of characteristic reactions in and around an autologous or a homologous musculofascial graft resided principally in tissues of the transplant. It also seemed reasonable to assume that each type of graft possessed a common factor which stimulated tissues of the host to penetrate and vascularize the graft. It was further assumed that homologous musculofascial grafts possessed one or more additional factors which excited an inflammatory reaction to accompany stromal organization of the transplant by tissues of the recipient. Theoretically, such factors might be regarded as having different thermal stabilities. Hence, the following methods for thermal treatment of musculofascial grafts before implantation were used with the idea that a range of thermal exposure might be defined within which the homologous graft might retain the stimulus to stromal penetration and lose the stimulus responsible for the inflammatory reaction by tissues of the host.

Methods

Methods of Transplantation

Young male New Zealand rabbits of the same strain and weighing 2-3 kg. were used. The surgical procedure for transplantation has been previously described.[1] Masses of erector spinae muscle covered with fascia and averaging 2×1.5×1.5 cm. were excised and placed in sterile, heavy-duty, round-bottom, centrifuge tubes of 90 ml. capacity. The tissues in the tubes were then exposed to various temperatures for specified lengths of time and subsequently transplanted. The procedure of transplantation was varied for control and experimental purposes. The animals were killed by cisternal puncture and injection of a 1% solution of procaine hydrochloride two to four weeks after transplantation. The skin was removed over the sites of transplantation, and the animals were fixed in 10% formalin for 72 hours. Following fixation, the transplants were serially cut into thin blocks in a plane perpendicular to the vertebral column. The

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thin blocks were prepared for microscopic study by embedding in paraffin and staining the sections with hematoxylin and eosin.

Methods of Freezing and Heating of Grafts

A. Liquid Nitrogen at —190 C.

1. Immersion in Liquid Nitrogen: Musculofascial transplants were excised and placed immediately in centrifuge tubes containing liquid nitrogen. After immersion in liquid nitrogen for 5 minutes, the transplants were removed and allowed to thaw at room temperature for 25 minutes. The transplants were then placed in the recipient as desired.

2. In Air with Immersion of Container in Liquid Nitrogen: Musculofascial transplants were excised and placed upon metal screens in tubes previously described. The transplants were placed so as to avoid their touching the sides of the tubes. The tubes were then immersed in a bath of liquid nitrogen in a Dewar flask for 30 minutes. Each tube was then removed from the liquid nitrogen and 10 ml. of sterile isotonic saline at room temperature was added. Tubes were transferred to a water bath at +37 C for 10 minutes. The transplants were then removed from the tubes and implanted in the animals.

B. In Air with Immersion of Container in a Mixture of Dry Ice (Carbon Dioxide Snow) and Acetone.

A sludge of dry ice and acetone was prepared to maintain a temperature of —78 to —80 C. Transplants were excised and placed on metal screens in tubes, as previously described. The tubes were then placed in the sludge. A thermometer centered in an empty tube was used to study the rate of temperature drop. After 30 minutes the tubes were removed and, after addition of isotonic saline, were heated for 10 minutes at +37 C. After warming, the grafts were implanted as desired.

C. In Air with Container in a Refrigerator.

Transplants were excised and placed in sterile tubes, as previously described. The tubes and a thermometer in a similar tube were then placed in the refrigeration compartment of an International Refrigerated Centrifuge to remain for 30 minutes to 72 hours at temperatures of from —16 C to +8 C, in different experiments. After this, 10 ml. of sterile isotonic saline was added to each tube. The tubes were then placed in a constant temperature bath at +37 C for 10 minutes. The transplants were then implanted in the animals.

D. In Air with Immersion of Container in a Heated Constant-Temperature Bath.

Grafts were excised and placed in tubes, as previously described. The tubes were placed in a constant-temperature bath at temperatures of +56 to +64 C for 30 minutes. After removal, 10 ml. of sterile isotonic saline was added to each tube, and the tubes were placed in another constant-temperature bath at +37 C for 10 minutes. The transplants were then removed and implanted as desired.

E. In Air with Container Immersed in Sludge of Ice Chips, 95% Alcohol, and Aqueous Sodium Chloride.

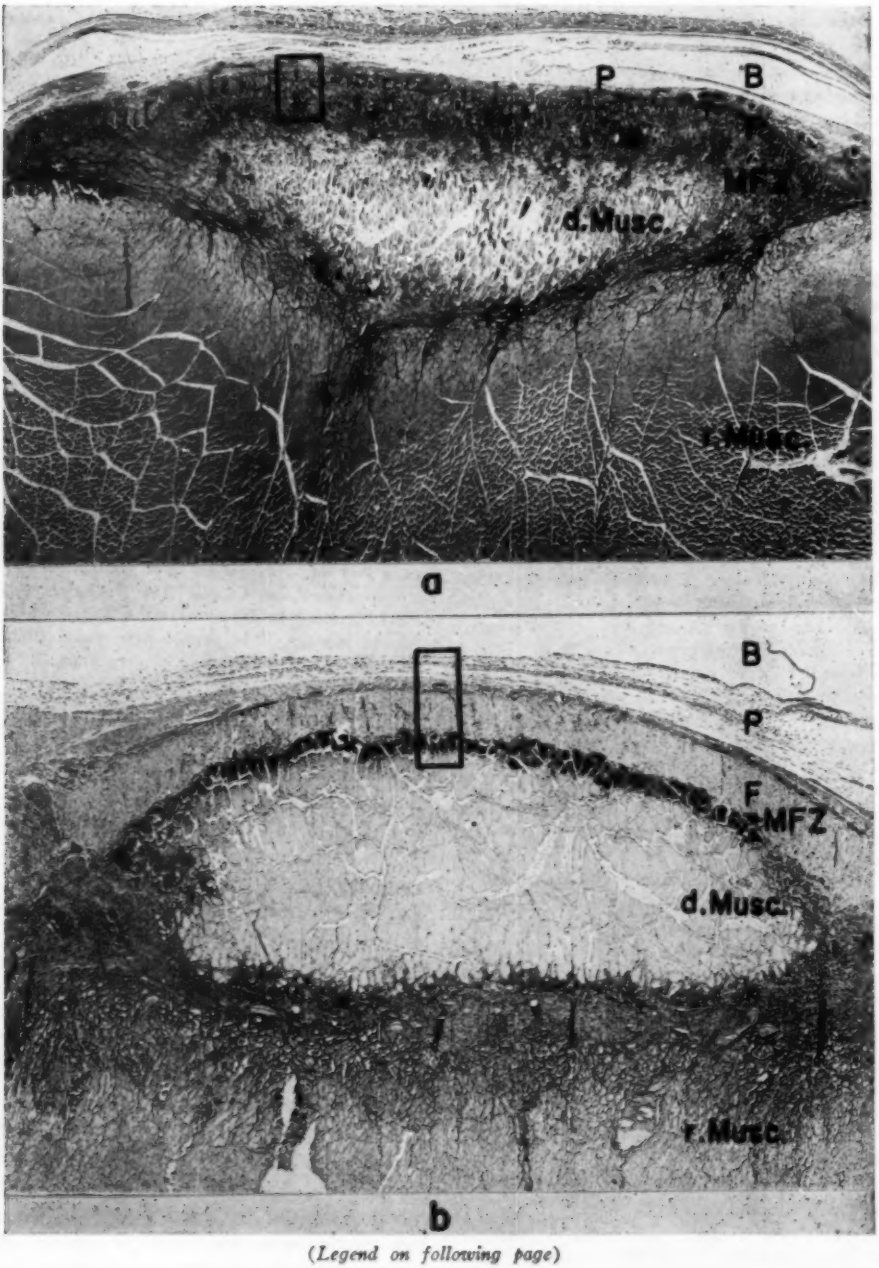
A sludge containing ice chips, 95% alcohol (optional for lower temperatures), and aqueous sodium chloride was prepared in Dewar flasks to obtain temperatures of 0 to —22 C. Musculofascial transplants were excised, cooled for 1-30 minutes, and handled as previously described in Method B.

F. Grafts in Glycerin in Container Immersed in Dry Ice and Acetone.

Musculofascial grafts were excised and placed on metal screens in tubes containing 20 ml. of 40%, 20%, or 5% glycerin in Ringer's solution. The tubes were allowed to stand at room temperature for 30 minutes to allow glycerin to diffuse into the tissues. The tubes were then placed in a Dewar flask containing a dry ice-acetone mixture at —78 to —80 C. After 30 minutes the tubes were removed and placed in a constant-temperature bath at +37 C for five minutes. After thawing, each graft was placed in a 250 ml. Erlenmeyer flask containing sterile isotonic saline for rinsing. The flasks were then placed in the constant-temperature bath at +37 C for 10 minutes. The grafts were then removed from the flasks and sutured into the animals.

Results

Previous studies of the gross appearance of normal autologous and homologous musculofascial grafts showed that they were healed in place by the end of the first week. A vascular network was visible in the pannus over the grafts. During the second and third weeks after transplantation the musculofascial zone increased greatly in breadth in both types of grafts. Subsequently, the grafts steadily decreased in size until by the end of the fourth and fifth weeks very little remained. Microscopic study of the region within and around each graft permitted sharp distinction between an autograft and a homograft.[1] The region of particular interest was directly beneath the fascia of the transplant (Fig. 1*A*, *B*). Normally, in unresected erector spinae muscle, the fascia was observed to consist of bundles of collagen between which a few fibrocytes were located. The fascia was



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avascular and in direct apposition to skeletal muscle fibers, with little actual space between them (Fig. 2*A*).

Two weeks following transplantation, the tissues of an autograft or a homograft were still distinct from those of the graft bed (Fig. 1*A*). A bursa and pannus had formed over each transplant. The musculofascial zone was increased in breadth, largely as the result of uniform absorption of subfascial muscle of the grafts. The formation and organization of the musculofascial zone of both types of grafts were of interest. An autograft at 2 weeks of age was covered with a pannus of vascularized granulation tissue derived from tissues of the host (Fig. 2*B*). The fascia was usually edematous and thickened, presumably due to retraction. A rich vascular network arising from the pannus had grown through the fascia to terminate in the musculofascial zone. This was now broad and expanded by additional granulation tissue, which had grown in from beneath the fascia at the margins of the graft. Penetration of the host's granulation tissue into the interior of the muscular portion of the grafts did not occur.

A similar sequence of events was noted in the musculofascial zone of homografts, though there was interference with the normal pattern of organization by inflammatory and other reactions which were features of homologous host-graft tissue interactions (Figs. 1*A*,2*C*).

Autologous and homologous musculofascial grafts treated as in the accompanying Table, at temperatures of −13 to −190 C and of +56 to +65 C, resembled one another grossly up to several weeks after implantation. The fascia remained pearly-white in appearance, except at its periphery, where vascularized granulation tissue was observed. A slightly opaque, thin film of tissue was noted directly overlying the transplanted fascia. These grafts also showed a retardation in the rate of absorption. Even after four weeks much of each type of graft was still easily distinguishable, having apparently resisted mechanisms of active resolution and organization, which affect normal untreated transplants (Fig. 1*B*).

Microscopic study of autologous and homologous transplants exposed to temperatures of −13 to −190 C and of +56 to +65 C showed a pattern of organization and absorption different from that of normal, untreated grafts. Autografts previously exposed to −190 C were well healed in place at 2 weeks of age (Fig. 3*A*). A bursa and an avascular pannus developed

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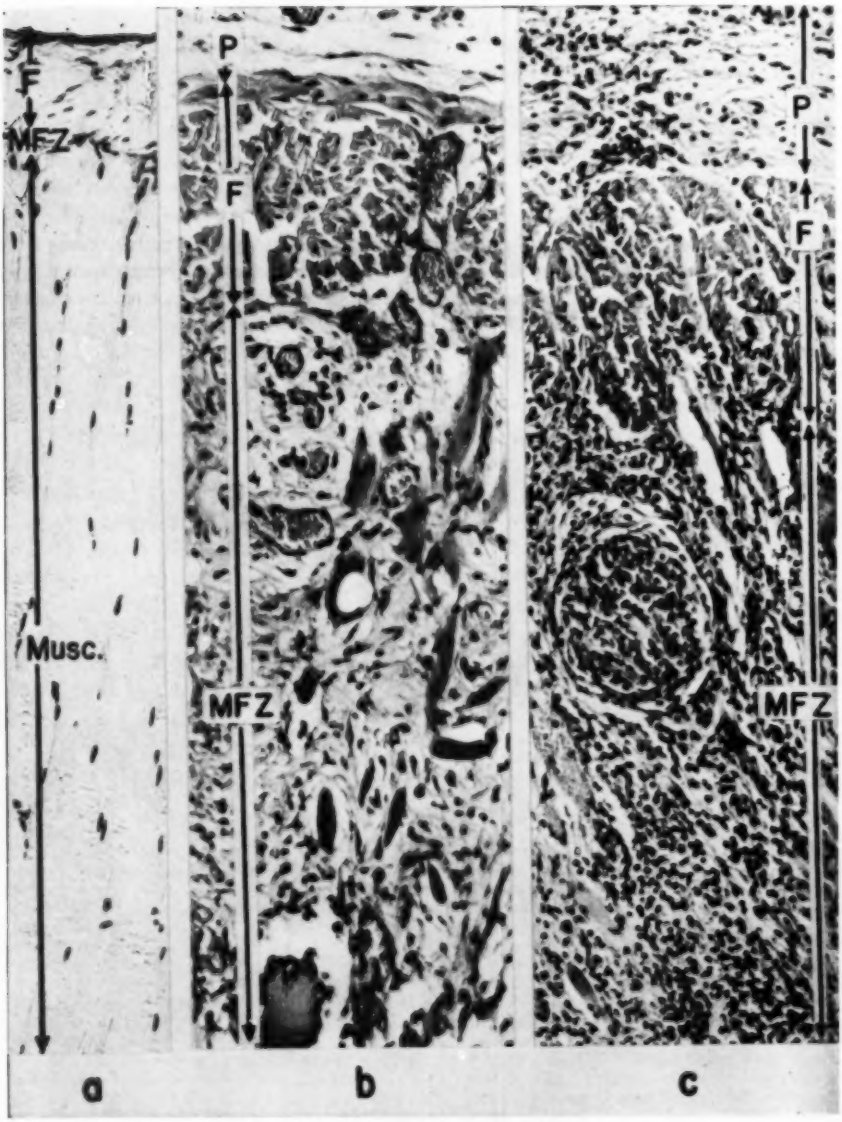
Fig. 1.—*A*, a low-power photomicrograph of an homologous musculofascial transplant, 2 weeks of age. A bursal space (*B*) has been formed between the superficial fascia of the host and the transplant. The pannus (*P*) consists of vascularized granulation tissue which has grown over the transplant to complete the floor of the bursal space. Beneath the pannus is the transplanted fascia (*F*), which has been permeated by blood vessels from the overlying pannus. The musculofascial zone (*MFZ*) created by the absorption of degenerating muscle (*d. Musc.*) of the graft is dark because of the dense infiltration of lymphocytes characterizing the homologous tissue reaction. The recipient's muscle is separated from the graft by newly formed granulation tissue. A photomicrograph of higher magnification from the region of the graft designated by the rectangular insert is shown in Figure 2*C*. This region affords features which allow distinction to be made between untreated autologous and homologous musculofascial transplants in rabbits. Magnification × 20.

B, low-power photomicrograph of an homologous musculofascial graft, 4 weeks of age. This resected graft had been exposed to a temperature of −190 C for 25 minutes prior to transplantation. The bursal space (*B*) lies above the recently vascularized pannus (*P*), which is free of inflammation. The fascia (*F*) of the graft is thickened, due to retraction, and is permeated by blood vessels only at the resected margins, where vascularized granulation tissue is both above and below the fascia. The musculofascial zone (*MFZ*) is very narrow. The skeletal muscle fibers beneath the fascia show deep staining with hematoxylin, due to pathologic calcification. The absorption of skeletal muscle of the graft is taking place principally from the lateral and inferior aspects of the graft bed, where normal granulation tissues are being formed. The recipient's skeletal muscle occupies the lower one-third of the illustration.

Photomicrographs (Figs. 3, 4, and 5) are enlargements of fields in various grafts selected in the region designated by the rectangle. Magnification × 20.



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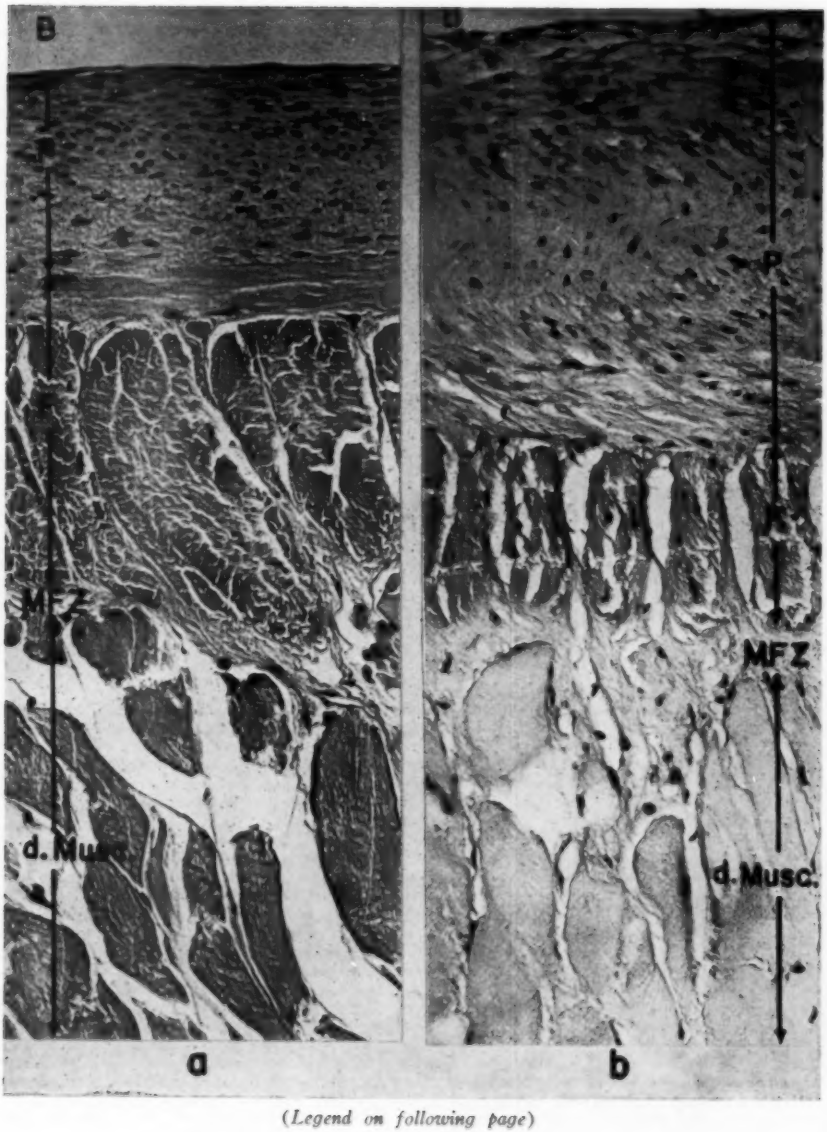
Effects of Wide Range of Thermal Pretreatment of Autologous and Homologous Grafts in Vitro on Nature of the Host-Graft Tissue Reaction Following Implantation.

Thermal Exposure of Transplants Before Implantation			Types of Musculofascial Transplants							
			Autologous				Homologous			
Method	Temp., Degrees (C)	Time, Min.	Age, Days	No. of Grafts	Type of Host-Graft Tissue Reaction		Age in Days	No. of Grafts	Type of Host-Graft Tissue Reaction	
					Autologous	Null			Homologous	Null
D	+56	27	14	8	0	+	..	..	..	..
D	+65	19	21	1	0	+	21	4	0	+
A1	−190	5	14	11	0	+	14	3	0	+
A2	−190	25	14	2	0	+	21	2	0	+
A2	−190	25	21	4	0	+	28	2	0	+
B	−78	25	14	11	0	+	14	2	0	+
B	−78	25	21	8	0	+	21	2	0	+
F 40%	−78	20	14	2	0	+	−	−	−	−
F 20%	−78	20	..	..	−	−	14	4	0	+
F 5%	−78	20	..	..	−	−	21	4	0	+
E	−22	20	..	..	−	−	14	2	0	+
E	−20	30	..	..	−	−	14	4	0	+
E	−18, −20	20	14	5	0	+	14	5	0	+
E	−20	1	14	4	+	0	14	4	+	0
C	−16	1	14	7	5	2	14	12	10	2
C	−15	1	..	..	−	−	14	2	+	0
E	−13	20	14	2	0	+	−	−	−	−
C	−11, −12	10	7	2	+	0	7	2	+	0
C	−11, −12	10	14	10	+	0	14	7	+	0
C	−10	4320	..	..	−	−	21	2	0	+
C	−10	4320	..	..	−	−	28	2	0	+
C	−8	15	14	11	+	0	14	10	+	0
E	0, −5	20	14	16	+	0	14	4	+	0
E	0, −5	20	21	8	+	0	−	−	−	−
C	−3	4320	14	2	+	0	14	2	+	0
C	+6, +8	4320	..	..	−	−	21	2	+	0
C	+6, +8	4320	..	..	−	−	28	2	+	0

Fig. 2.—*A*, medium-power photomicrograph showing the normal relationship of fascia to the erector spinae muscle in the rabbit. The fascia (*F*) consists of heavy bundles of collagen, between which there are a few fibrocytes. This tissue is void of blood vessels. Delicate bands of collagen pass from the fascia to ensheath the individual muscle fibers (*Musc.*). The musculofascial zone (*MFZ*) is avascular and is purely junctional in character. It is in this region where the most distinctive changes occur in the untreated musculofascial transplants. Magnification × 300.

B, medium-power photomicrograph of the musculofascial junction of an autologous graft, 2 weeks of age. The pannus (*P*) consists of the recipient's vascularized granulation tissue, which is fused with the surface of the transplanted fascia. Blood vessels from the pannus pass almost perpendicularly through the fascia (*F*) into the musculofascial zone (*MFZ*), which has been created by the absorption of skeletal muscle of the transplant. The musculofascial zone shows vascularization, collagen deposition, fibroblastic activity, and absorption of skeletal muscle in the absence of inflammation. Prompt vascularization of the fascia and the development of the musculofascial zone were constant features of the autologous tissue reaction to untreated grafts. Magnification × 300.

C, medium-power photomicrograph of the musculofascial junction of an homologous transplant, 2 weeks of age. The pannus (*P*) consists of vascularized granulation tissue of the recipient, in which cellular infiltration is noted. Vessels from the pannus have passed through the fascia (*F*) to terminate in the region of the musculofascial zone (*MFZ*). This region consists of vascularized granulation tissue in which lymphocytic infiltration, impaired collagen deposition, limited fibroblastic activity, and varying degrees of angiitis are encountered. This interference with the normal sequences of healing is characteristic of the tissue reaction to untreated homologous musculofascial grafts in rabbits. Magnification × 300.



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over the transplants. The fascia of the grafts stained poorly. It was mostly acellular and exhibited no blood-vessel penetration except at the periphery, where granulation tissues of the host were located on both sides of the fascia. The musculofascial zone did not change. There was no absorption of skeletal muscle from beneath the fascia, and no mesenchymal proliferation was encountered in this region. The absorption of skeletal muscle was taking place principally at the inferior and lateral aspects of the transplant, where vascularized granulation tissues of the host were penetrating the graft.

The pattern of organization and resorption of homologous musculofascial grafts exposed to temperatures of −13 to −190 C and of +56 to +65 C was similar to that of autografts. The treated homografts showed no tendency to elicit inflammation over prolonged periods of time (Figs. 1*B*, 3*B*). It was impossible to detect any difference in the host-graft reactions to autografts and homografts kept at −20 C for 20 minutes prior to implantation (Fig. 4*A*,*B*).

Musculofascial grafts which were kept at temperatures of between +8 and −5 C for 15-4320 minutes showed the pattern of organization and resorption characteristic of normal, untreated grafts, except that the homologous reaction was somewhat less intense and more focal in distribution in the musculofascial zone.

Grafts which were momentarily exposed to low temperatures showed the characteristic tissue reaction of untreated grafts (Fig. 5*A*,*B*). Grafts kept at −3 C for three days and then transplanted elicited the response typical of autologous and homologous transplants.

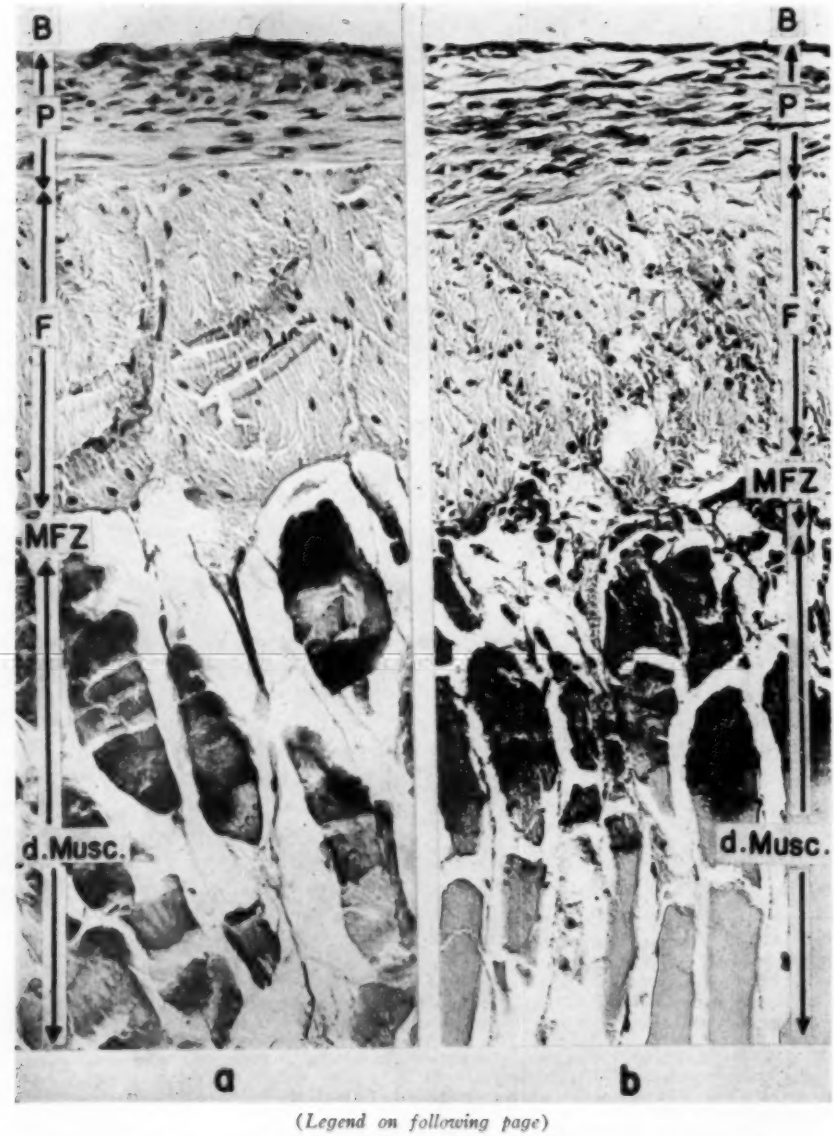
Comment

These studies were designed to determine whether fresh musculofascial grafts could be modified in vitro by thermal methods so that upon subsequent transplantation the factors in homologous grafts which excite homologous incompatibility reactions might be distinguished from factors in autologous grafts which elicit autologous compatibility reactions. The results have shown that these two types of tissue reactions are elicited by factors which have essentially the same susceptibility to thermal treatment in vitro. The susceptibility to thermal treatment indicated that the mere presence of autologous or homologous tissue proteins in a graft could hardly be held responsible for development of the characteristic reactions. Indeed, the tissue proteins, even after mild exposure to low or high temperatures, seemed capable of stimulating nothing more than an indolent host reaction, which was the same irrespective of the autologous or homologous nature of the graft.

The critical level of thermal exposure below which grafts lost the capacity to stimulate active host-graft interactions was in the region of −10 to −15 C for 20 minutes. This level corresponded closely with the level of hypothermal treatment required to eliminate the capacity for skeletal muscle

Fig. 3.—*A*, medium-power photomicrograph of the musculofascial junction of an autologous graft, 2 weeks of age, from the area of the rectangle in Figure 1*B*. This resected graft had been exposed to a temperature of −190 C for 25 minutes prior to transplantation. The empty space above the tissue is a part of the bursal space overlying the transplant. The Pannus (*P*) consists of a well-developed layer of avascular granulation tissue, which is in direct apposition with the fascia (*F*) of the transplant. This fascia has a reduced cellularity, stains poorly, and is also avascular. The musculofascial zone is practically nonexistent except for a delicate matrix of reticulum produced by the avascular granulation tissues of the recipient. The skeletal muscle of the donor (*d. Musc.*) shows retardation in absorption. Magnification × 300.

B, medium-power photomicrograph of the musculofascial junction of an homologous graft, 2 weeks of age. This graft was treated in the same way as the autograft shown in Figure 3*A*. The sequence of healing between the donor's and the recipient's tissues is identical with that encountered in the autograft (Fig. 3*A*). Homologous proteins in the graft altered by exposure to low temperature did not elicit a normal pattern of vascularization and had no tendency to induce an inflammatory response on the part of the host. Magnification × 300.



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to grow in tissue culture.[2] The critical level above which grafts lost the capacity to stimulate the interactions was not carefully worked out, but it was less than +56 C for 27 minutes. In either case, whether low or high thermal exposure was used, the result, so far as the host or graft was concerned, was about the same. These conditions of exposure are probably not far removed from those generally believed to be "lethal" for most adult mammalian cells, though the effect of low temperature, particularly, has not been entirely understood or agreed upon. It seems apparent that all adult cells are not equally susceptible and that at least some embryonic cells are very resistant to low temperatures under special conditions.* There are also many good reasons for believing that all functional enzymatic or other systems of cells are not equally susceptible to thermal exposure, some being more resistant than the cells themselves. Also, in this connection, some viruses which are intracellular inhabitants are more susceptible to thermal changes than the more complex mammalian cells.[5]

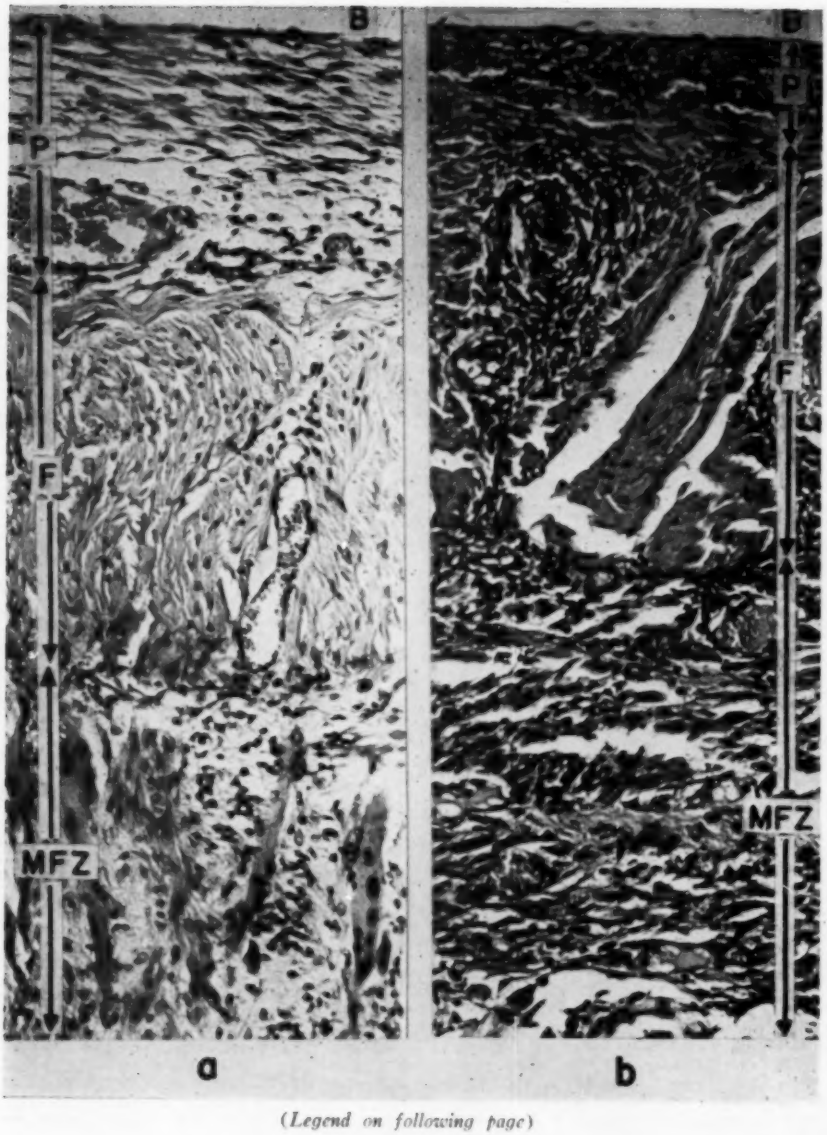
The present study has given no satisfactory definition of what is meant by "lethal" effects of thermal treatment. However, the data have shown that the indicated thermal treatment of grafts modified the tissues so that the host became unable, in a local reactive sense, to distinguish between autologous and homologous grafts. The over-all response of the host was the same for the two types of grafts, and this consisted principally of fibroblastic and collagenous encapsulation, with slow permeation and resorption of the graft. This indicated that stimuli of both types of modified grafts to host-graft interactions were probably of minimal and equal intensity. Under such conditions, the homologous graft might be regarded as a relatively ineffective antigen, even though, theoretically, the components of the graft were potent antigens. Perhaps, the thermal treatment had in no way affected the exciting factors in either type of graft, but had only interfered with the sequence of reactions governing the accessibility of the factors to the host or vice versa.

When modifications of the grafts were produced by a little less than critical thermal exposure, characteristic autologous and homologous host-graft interactions regularly developed. The over-all response of the host consisted of fibroblastic proliferation and collagenous encapsulation with a highly vascularized stroma, which promptly permeated the graft. The richly vascularized pannus and the conspicuous vascular penetration of the dense fascia of the graft seemed to be a response to stimuli arising from the subfascial zone of the graft. These stimuli, in turn, seemed to arise from mesenchymal cells of the graft, which responded to the host's environment by proliferation, production of intercellular materials, and limited differentiation in the protected subfascial environment. Furthermore, the intensity of the local host-graft interactions was directly proportional to the extent of admixture of proliferating vas-

* References 3, 4.

Fig. 4.—*A*, medium-power photomicrograph of the musculofascial junction of an autologous transplant, 2 weeks of age. This resected autograft had been exposed to a temperature of —20 C for 20 minutes prior to transplantation. The empty space (*B*) above the tissue is a part of the bursal space overlying the graft. The pannus (*P*) is well developed and consists of avascular granulation tissue, which is adherent to the fascia (*F*) of the graft. The fascia shows moderate cellularity but is conspicuously avascular. The musculofascial zone has failed to form and is represented by spaces created by the retraction of skeleton muscle fibers away from the fascia. The fibers of the donor's muscle (*d. Musc.*) show dark regions consistent with pathologic calcification. Magnification × 300.

B, medium-power photomicrograph of the musculofascial junction of an homologous graft, 2 weeks of age. This resected homograft had been exposed to —20 C for 20 minutes prior to transplantation. The tissue reaction is identical with that encountered in the autograft (Fig. 4*A*). Exposure of homologous and autologous musculofascial grafts to a temperature of —20 C for 20 minutes prior to transplantation caused changes which led to a similar pattern of reaction of tissues of the host to both types of grafts. Magnification × 300.



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cularized tissues of the host with the comparable tissues of the graft. Therefore, the analysis of tissue reactions in and around autologous and homologous grafts eventually may be made only through an inquiry into factors concerned with the healing process as it involves two merging activated tissue systems. The mutual participation of the tissue of the host and the tissue of the graft may lead to different patterns of reaction, depending upon the status of the graft, and presumably also upon the status of the host. If the appropriate status for each can be found, successful transplantation of homologous tissues may become more practical than is currently the case.

The sequences of healing between graft and host may be attributable to several factors which seem to reside within the tissues of the graft. The stimulus to the host's stromal and vascularizing potential is a factor which normal autologous and homologous musculofascial grafts possess. Beyond this, the homograft must possess an additional exciting factor responsible for the train of events culminating in the inflammatory reaction and the commonly associated interference with its newly established blood supply.

A conspicuous feature of these host-graft tissue reactions was that the intensity of the autologous or homologous reaction was directly proportional to the intermingling of vascularized stroma of the host with proliferating cells of the graft. Previous experience had indicated that anything which reduced the amount of vascularized stroma from the host, the number of proliferating cells of the graft, or the degree of intermingling of these elements reduced the intensity of the autologous or homologous reaction. In these experiments, approximate amounts of thermal treatment required to eliminate the homologous reaction have been defined and proved to be the same as those required to eliminate the autologous reaction. The extent to which this thermal treatment interfered with various cellular activities was unknown except that the required treatment was essentially the same as that which eliminates the capacity of the principal cells of the graft to multiply in tissue culture.[2] It is doubtful whether the loss of this capacity alone can be regarded as evidence of loss of viability of the principal cells. The relation of viability of cells in the graft to the host-graft interactions may properly serve as a subject for further study.

These observations have led to the conclusion that the most profitable course to follow in searching for the source of stimuli which give rise to the characteristic reactions is to examine the action of diffusible products of actively multiplying admixtures of cells of the graft and host. This implies the existence of some mechanism by which reactions of the tissues of the host, and perhaps those of the graft as well, are mutually elicited by such diffusible products to the advantage or disadvantage of both.

Summary

Previous histological studies of host-graft tissue interactions in and around autologous

Fig. 5.—*A*, medium-power photomicrograph of the musculofascial junction of an autologous graft, 2 weeks of age. This resected graft had been momentarily exposed to a temperature of —20 C. The empty space (*B*) above the tissue is a part of the bursal space overlying the graft. The pannus (*P*) is well developed and contains numerous normal vascular channels which have readily penetrated the fascia (*F*) into the musculofascial zone (*MFZ*). This zone consists of absorbing skeletal muscle within a matrix of normal granulation tissue. This pattern of vascularization and resorption of the graft represents the usual autologous tissue reaction and is unaffected by the momentary exposure to —20 C. Magnification × 300.

B, medium-power photomicrograph of the musculofascial junction of an homograft, 2 weeks of age. This graft had been momentarily exposed to a temperature of —20 C prior to transplantation. The pattern of tissue reaction is the same as in the autograft (Fig. 5*A*) with the exception of the diffuse inflammatory reaction and angiitis accompanying the vascularized stroma of the host in and around the graft. Untreated homologous musculofascial transplants elicit an identical reaction. Magnification × 300.

or homologous musculofascial transplants in rabbits indicated that the intensity of the interactions depended on the absolute and relative amounts of a vascularized mixture of proliferating mesenchyme from the two sources. In addition, the intensity of the homologous interactions seemed to depend upon the concentration of additional factors elaborated by the mixture of proliferating tissues. It was assumed that the existence of such theoretical factors might be more clearly defined if grafts were exposed to a broad range of low and high temperatures prior to implantation.

The results of these experiments disclose that exposure of grafts for 20 minutes to temperatures below −20 C, or for 27 minutes at +56 C, eliminated all factors responsible for characteristic autologous and homologous host-graft interactions. In their stead there is a new form of host-graft interaction, which is identical for autologous and homologous transplants. This is defined as a null interaction. It is characterized by negligible proliferation of cells and retarded resorption of tissues of the graft, coupled with avascular encapsulation and indolent avascular penetration of the graft by stromal tissues of the host. These observations lead to the conclusion that the future search for factors responsible for incompatibility reactions might be conducted profitably by examining the local products of an admixture of vascularized proliferating mesenchyme arising from the dual sources.

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The Pancreas of Cortisone-Treated Rabbits

A Pathogenic Study

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In previous work,[1] it was found that rabbits treated with diabetogenic doses of cortisone developed in the pancreas a diffuse proliferation of small ductular elements, in addition to the other lesions which are usually associated with experimental diabetes. At that time it was shown that these proliferative changes were more dependent upon the length of therapy than upon the severity of the induced diabetes. This, and the fact that similar changes have not been reported in other forms of experimental diabetes, would suggest that this diffuse ductular proliferation is probably not related pathogenetically to insulin insufficiency, hyperglycemia, or obstruction due to glycogen infiltration (hydropic change) in ducts. It was therefore considered that the ductular proliferation was not related to the diabetic syndrome per se but, rather, was associated with some other action of cortisone.

Pancreatic lesions characterized by prominence of ductular elements have been described in vitamin A deficiency,[2] malnutrition with marked nicotinic acid deficiency,* and kwashiorkor,[5] which is considered primarily to be a protein depletion syndrome with associated cyanacobalamin (vitamin B_{12}), methionine, and choline insufficiency. A similar lesion has also been reported in chronic wasting diseases and uremia[6] and after injection of bacterial toxins.[7]

Adrenal overactivity has been stated to result in a metabolic and a pathologic state akin to that observed in malnutrition[8] and causes disturbances in the metabolism of cyanacobalamin U.S.P.† and vitamin A.‡ In addition, hyperadrenocorticalism increases the susceptibility to infection.§ This was verified by experiments in which several cortisone-treated rabbits died of Pasteurella pneumonia, whereas untreated animals in the same room remained well.[1]

In view of the above, a series of experiments was designed to test the possibility that weight loss, vitamin deficiency, or infection might account for the ductular lesion of cortisone-treated rabbits. In addition, because of the intimate relation between these lesions and diabetes, and because of their similarity to those observed in obstructive conditions in the pancreas, alloxan-diabetic and duct-ligated rabbits were also studied.

Material and Methods

This study was carried out on 132 New Zealand white rabbits of either sex. All animals were placed in individual metabolic cages and, except for Groups E and F, were allowed continuous free access to water and to weighed amounts of Purina rabbit chow. Each rabbit was weighed biweekly. For Groups B, C, and D, 24-hour urine specimens were collected three times weekly in clean vessels, to which 1 to 2 cc. of toluene had

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* References 3 and 4.

† References 9-11.

‡ References 12, 13.

§ References 14-16.

been added. Urine glucose was determined by Benedict's quantitative method.[17] Samples for blood glucose were obtained in duplicate from the ear veins, using micropipettes. Blood sugar was determined by a modification of the Folin-Wu micromethod.[18] Blood sugar determinations were done biweekly for Groups B, C, and D. For Group G the blood glucose was determined biweekly for the first month and once weekly thereafter. For the animals of Group B, which were killed prior to the 17th day, the urine and blood sugar determinations were done daily. Animals were killed by air embolization. Specimens from both the duodenal and the splenic portion of the pancreas were fixed in Zenker-formol and stained with the Masson trichrome, the chrome-alum-hematoxylin,[19] and the aldehyde-fuchsin methods.[20] In addition, glycogen was demonstrated by the periodic acid-Schiff technique; counterstained with iron hematoxylin, orange G, and aniline blue, and controlled by diastase digestion.[21] The animals were divided into seven groups.

Group A: *Morphologic Control.*—Pancreases from 20 untreated rabbits, weighing from 2.5 to 6 kg., were used.

Group B: *Cortisone and Antibiotics.*—Forty-three rabbits, weighing from 2 to 5.8 kg., each received 5 mg/kg. daily of cortisone acetate ‖ intramuscularly in the thigh for the first two weeks and then 10 mg/kg. daily. Each animal also received intramuscularly 150,000 I. U. of procaine penicillin G U. S. P. and 200 mg. of dihydrostreptomycin biweekly. Two animals each were killed at one and two days after the starting of cortisone. Three each were killed at 3 and 4 days, and two each were killed at 6, 8, 10, and 17 days. Biopsy specimens of the pancreas were obtained under pentobarbital (Nembutal) anesthesia from 17 of the remaining rabbits at 30 days and from the last 8 at 45 days.

Group C: *Cortisone, Antibiotics and B Vitamins.*—Six rabbits, whose weight varied from 4.4 to 5.5 kg., received 12.5 mg. of cortisone intramuscularly daily for the first week. At that time the dose was increased to 25 mg. daily. Two weeks later this was further increased to 37.5 mg. Each rabbit also received 150,000 I. U. of procaine penicillin G and 800 mg. of chloramphenicol U. S. P. (Chloromycetin) biweekly. In addition, a daily vitamin supplement which contained 3 mg. of cyanacobalamin, 15 mg. of thiamine hydrochloride, 1.5 mg. of riboflavin, 35 mg. of nicotinic acid U. S. P., 2.5 mg. of pyridoxine, and 2.5 mg. of calcium pantothenate were given subcutaneously. This group of animals was maintained as long as survival permitted, up to 60 days. One animal each died at 31, 38, 42, 46, and 53 days. The sixth animal was killed at 60 days.

Group D: *Cortisone, Antibiotics and Vitamins A, C, and D.*—Five rabbits, whose weight varied from 4.5 to 5.5 kg., each received 25 mg. of cortisone daily for two weeks and then 50 mg. daily. In addition, each animal received orally by dropper 0.6 cc. of Trivisol,¶ which contains 5000 I. U. of vitamin A, 1000 I. U. of vitamin D, and 50 mg. of ascorbic acid. This group also received penicillin and chloramphenicol, as for Group C. One animal was killed at 21 days; three at 28 days, and one at 36 days.

Group E: *Partial Starvation.*—Six rabbits, whose weight varied between 2.7 and 3 kg., each received 50 gm. of food pellets on alternate days for the first 18 days and 25 gm. of pellets on alternate days thereafter. One animal died at 21 days; two were killed at 21 days; one, at 28 days, and one, at 38 days. The remaining animal died at 42 days.

Group F: *Complete Starvation.*—Ten rabbits, weighing between 1.6 and 4 kg., were deprived of all food but were allowed free access to water. When the animals began to show signs of severe weakness, the blood sugar was determined every three to four hours, and they were killed when the glycemic level fell below 50 mg/100 cc. One animal each died at 13, 17, 18, and 42 days, and one each was killed at 19, 23, 25, 28, 31, and 48 days.

Group G: *Alloxan Diabetes.*—Thirty-six rabbits, weighing between 2 and 5 kg., were given a single intravenous injection of 150 mg/kg. of alloxan monohydrate (Eastman Kodak Company), as a freshly prepared 5% solution in distilled water. Eleven of the animals died at various intervals and were not used for histologic studies. The remaining 25 were killed, as follows: four after 15 days, three at 3 months, five at 3½ months, three at 4 months, four at 5 months, and one at 7 months.

Group H: *Duct Ligation.*—The main pancreatic duct of six rabbits, weighing from 1.8 to 2.6 kg., was cut between ligatures under pentobarbital anesthesia. At the time of operation, a biopsy specimen of the duodenal portion of the pancreas was obtained. One animal each was killed at 1, 2, 7, 14, 21, and 50 days after duct ligation.

Results

Controls (Group A).—The intercalated ducts were not prominent in the normal

‖ Cortisone acetate was supplied through the courtesy of Dr. C. H. O'Donovan, of the Upjohn Company, Kalamazoo, Mich.

¶ Mead Johnson & Company, Ltd., of Canada.

pancreas. The cells of these ductules were small, pale-staining, and elongated in the direction of the lumina. The connection of the ductules to the islets was only occasionally apparent, and they were only infrequently seen within islets. The islets themselves were compact and round or oval, with a regular outline (Figs. 1 and 2).

On some occasions, the normal rabbit pancreas showed focal areas, which consisted of proliferating and dilated ductules with atrophic acini and moderate interacinar fibrosis. The islets present in these areas may appear normal (Figs. 3 and 4).

Cortisone-Treated Rabbits (Groups B, C, and D).—Since the responses of the three groups to therapy were similar, they will be described together. The animals seemed to tolerate the cortisone quite well, and very few died spontaneously before six weeks. Toward the end of this period, however, most animals became debilitated. Vitamin supplements apparently did not influence the clinical course.

In general, all rabbits showed an initial weight gain varying from 150 to 250 gm., after which the weight tended to decline and reached the starting values in 10 to 25

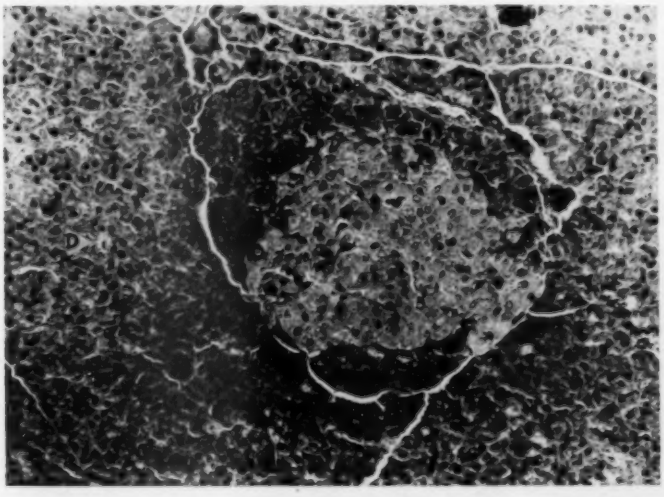

Fig. 1.—Area of untreated rabbit pancreas, showing the compact, comparatively regular outline of the islet, with sparsely distributed ductular elements (*D*). Masson's trichrome stain; reduced to 90% of mag. × 200.

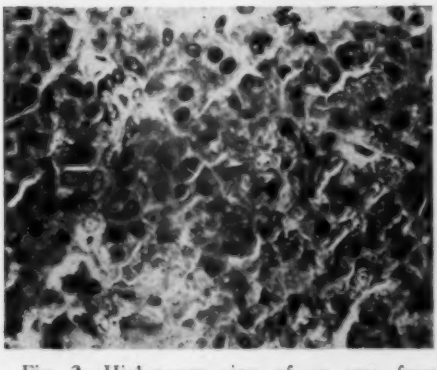

Fig. 2.—High-power view of an area from Figure 1, showing intercalated ducts with flattened epithelium and elongated nuclei interspersed among the acini. Masson's trichrome stain; reduced to 90% of mag. × 400.

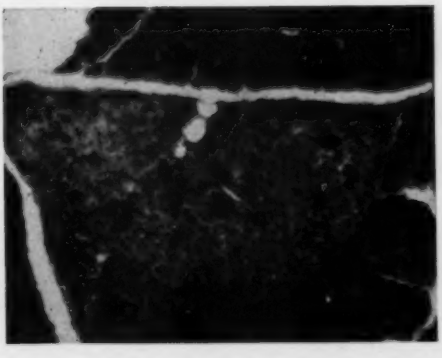

Fig. 3.—Untreated rabbit pancreas, illustrating a focal area of proliferating and dilated ductules with atrophic acini and normal-appearing islet. Masson's trichrome stain; × 84.

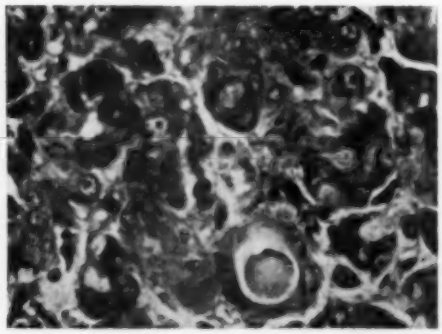

Fig. 4.—High-power view of an area from Figure 3, showing the proliferating and dilated ductules, some of which contain inspissated material. This is similar to what may be seen in the duct-ligated pancreas of seven days and also to some areas in the pancreas of cortisone-treated rabbits. Masson's trichrome stain; reduced to 90% of mag. × 400.

days. Those that survived longest showed a reduction in body weight which in many instances was 20% to 30% of the initial level. The degree of diabetes which developed was variable. The urine sugar ranged from a trace to 15 gm. in 24 hours. In some animals there were many days in which no glycosuria was present. The blood sugar concentration ranged from 150 to 500 mg. per 100 cc. and tended to remain more constantly elevated than the urine sugar. However, there was a fair correlation between the glycemic level and the urinary glucose output.

Morphologically, there were three types of lesions in the pancreas.

First Type: The first group of lesions consisted of degranulation and glycogen infiltration (hydropic change) of beta cells, as well as glycogen infiltration of duct cells. The beta-cell degranulation appeared as early as 48 hours after the cortisone treatment was started, whereas glycogen infiltration of beta cells and duct cells began about 4 days after cortisone administration. A detailed analysis of the beta-cell changes as they relate to the diabetic condition is to be the subject of a separate study.

Second Type: The second group of lesions, with which we are concerned, was characterized by diffuse proliferation of small ductules with associated intraislet duct proliferation and changes in size and shape of islets. In rabbits killed after 30 days of cortisone therapy, there was such an extensive hyperplasia and hypertrophy of the intralobular ductular elements (Fig. 5) that, when looked at under low-power magnification, these structures could be mistaken for small irregular islets. This was more noticeable because the beta cells were usually severely degranulated and the acinar tissue was mostly intact. These changes

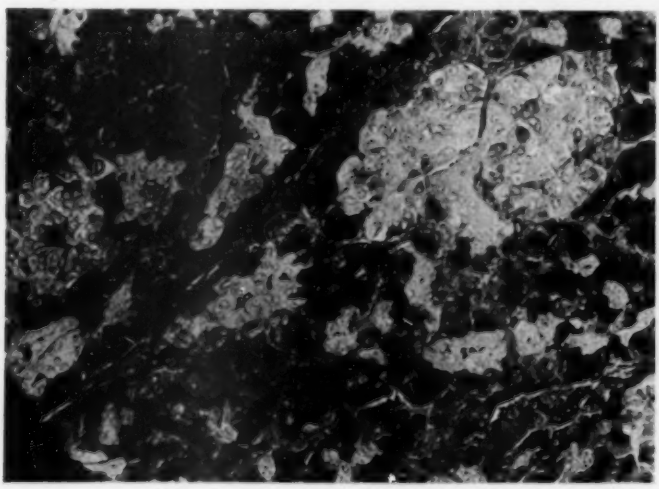

Fig. 5.—Pancreas of a rabbit treated for 30 days with cortisone, showing the marked proliferation of ductular elements (D), the lumen of which contains inspissated material, with comparatively normal-appearing acinar tissue. Compare with Figure 1, at the same power. There is also present an irregularly shaped islet in intimate contact with hyperplastic duct epithelium. Most beta cells are degranulated, and few show hydropic change. Because beta cells are markedly degranulated, the proliferating duct cells may be confused with small islets. Aldehyde-fuchsin; reduced to 90% of mag. × 200.

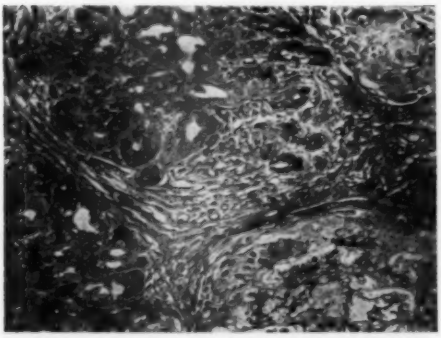

Fig. 6.—Pancreas of rabbit treated with cortisone for 30 days, showing an area of proliferating ductules with some atrophic acinar tissue and moderate fibrosis. There are also present an area of pancreatic fat necrosis, a few giant cells, and beginning organization in the necrotic region. Aldehyde-fuchsin; reduced to 90% of mag. × 100.

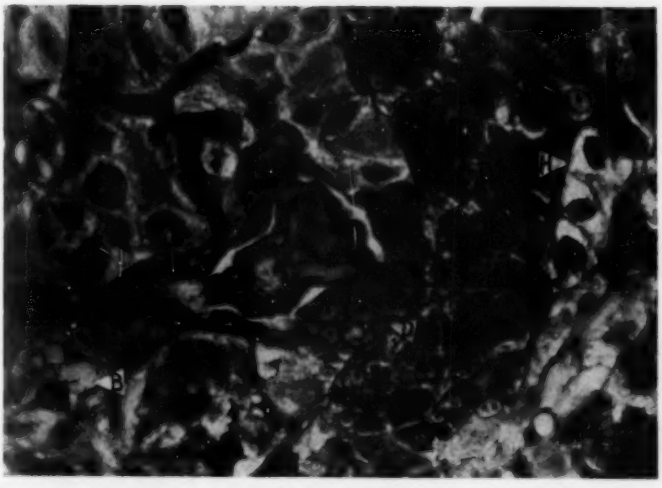

Fig. 7.—Pancreas of a rabbit treated for 30 days with cortisone, showing a proliferating dilated ductule within an islet. The lumen contains inspissated, laminated material. Hydropic degeneration of the duct epithelium (*H*) and of the beta cells (*B*) is seen. The acinar tissue is of average appearance. Aldehyde-fuchsin; reduced to 90% of mag. × 400.

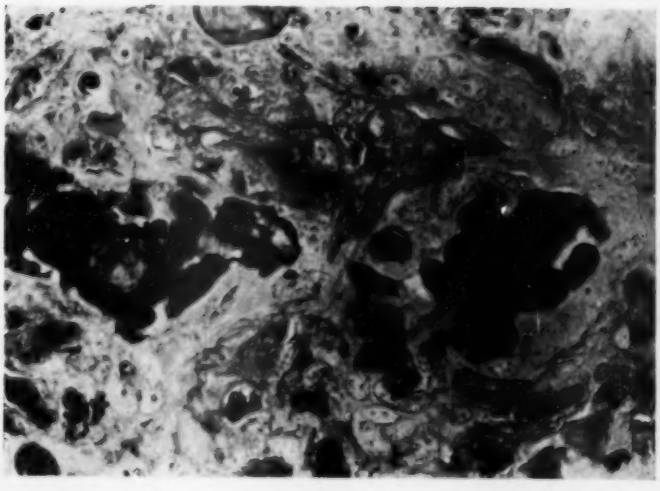

Fig. 8.—Same pancreas as that in Figure 5, showing two hyperplastic ducts with inspissated material in the lumen. Aldehyde-fuchsin; reduced to 90% of mag. × 400.

are striking when Figure 5 is compared with Figure 1. On the other hand, in some areas of the pancreas, the acinar tissue did show zones with profound regressive changes. These zones were composed almost entirely of proliferating ductules and atrophic acini with variable degrees of fibrosis (Fig 6).

Most proliferating ducts contained inspissated, and frequently laminated, material in their lumen (Figs. 5, 7, and 8). Proliferating ductules were often seen inside islets (Fig. 7). Frequently, these ducts became dilated, thus compressing islets in a manner similar to that described later in the pancreas of rabbits in which the main pancreatic duct had been ligated (compare Figure 7 with Figure 11). In contrast with the regular outline of the islets in the control animals, the islets of cortisone-treated rabbits were frequently irregular and lobated (Fig. 5). All the changes described were distributed throughout the pancreas, but were more prominent in some areas than in others.

Morphologic changes similar to those just described, but present in a much slighter degree, were seen in rabbits killed as early as eight days after starting cortisone treatment. In animals killed prior to this time, some abnormalities of the ductular system were found. However, because of their scarcity and basic identity with the focal lesions in the untreated animals, it was not possible to be sure that they were induced by the cortisone. However, after the 10th day all treated animals showed to a certain degree some of the ductular changes just described.

Third Type: The third group of pathological changes consisted of focal peripancreatic fat necrosis with pancreatic necrosis of adjacent acini and focal granulomas in the peripancreatic fat (Fig. 6). These areas of fat necrosis appeared grossly as minute flecks of opaque white and crusty-like material. Usually pinhead in size, they measured occasionally 3-4 mm. in diameter. Such grossly visible lesions were usually present in rabbits treated for 30 days or more, particularly in the region close to the duodenum.

Partial Starvation (Group E).—The animals gradually became emaciated and somnolent. The terminal weight loss was greatest in those animals that survived longest and varied from 500 to 1210 gm. The pancreases of these rabbits were indistinguishable from those of untreated animals.

Complete Starvation (Group F).—Terminally, the animals showed marked weakness and somnolence, with convulsions in the last 15 hours. During this last period, the blood sugar declined rapidly to values as low as 23 mg. per 100 cc. The weight loss varied between 700 and 1500 gm. The larger animals lost more weight and also survived longer. However, those that survived longest were accidentally fed on two occasions. The pancreas showed vacuolation of the acinar cells and loss of their basophilic substance. The islet tissue was unchanged. In one animal there were several focal areas where ducts were prominent. Some of these ductules were dilated, and in others their epithelium was slightly hypertrophic. However, no proliferation of ducts inside islets or abnormalities in the contour of the islets were noted.

Alloxan Diabetes (Group G).—Most animals showed a sustained marked hyperglycemia, ranging from 350 to 600 mg. per 100 cc., which developed within two days. In some instances there was complete resistance to the diabetogenic action of alloxan, or disappearance of the hyperglycemia for some time before death. With the exception of a few which lost weight toward the end of the experimental period, most rabbits gained weight, some up to 25% of their initial values. In general, weight loss was associated with an intercurrent disease, such as pneumonia, brain abscess, or pyelonephritis. The pancreatic islets of the alloxan-diabetic animals were small and few in number and were comprised of alpha and delta cells and occasional degranulated or hydropic beta cells. Marked glycogen in-

filtration (hydropic change) was seen occasionally in the ductular system of the pancreas of animals which had been severely diabetic for only 17 days but was regularly present after 30 days. These ducts were often seen to be continuous with islets, and hydropic duct cells were frequently present inside islets. No hyperplasia of the ductular system was observed. Those rabbits which became normoglycemic for some time before death did not show glycogen infiltration. In these animals the islets were composed mostly of alpha and delta cells, with a few well-granulated beta cells. There were also small islets made of beta cells only.

Duct Ligation (Group H).—None of the rabbits showed a significant change in its blood sugar level or in its body weight during the experimental period. In general, the

Fig. 9.—Pancreas from a rabbit 21 days after duct ligation, showing lobules composed of proliferating and moderately dilated ductules, dedifferentiated acinar tissue, and moderate fibrosis. In addition, there is present an area of pancreatic fat necrosis. Masson's trichrome stain; reduced to 90% of mag. × 34.

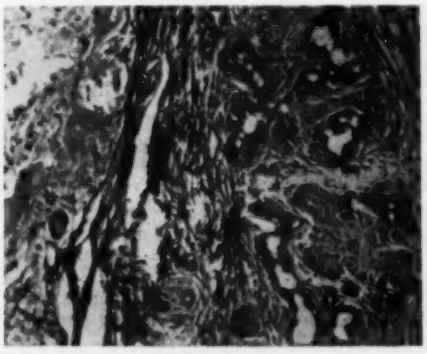

Fig. 10.—Higher-power view from an area of Figure 9, showing the histologic details. Compare with Figure 6. Masson's trichrome stain; reduced to 90% of mag. × 100.

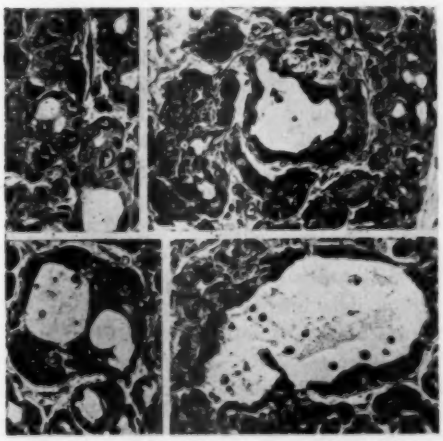

Fig. 11.—Composite picture of different areas from the pancreas of a rabbit seven days after duct ligation, showing ductular proliferation both in acinar and islet tissue. In the latter, there is marked ductular dilatation and the islets are being compressed and destroyed. Masson's trichrome stain; reduced to 90% of mag. × 200.

pancreatic stroma of all ligated animals showed a diffuse interstitial inflammatory reaction, which was acute and subacute in the early stages and chronic in animals killed after the 14th day. From the second day on there were focal areas of pancreatic necrosis and of peripancreatic fat necrosis. In addition, focal granulomas were present in the peripancreatic fat. After the 14th day there was progressive diffuse fibrosis, which was maximal in the rabbit killed at 51 days. The parenchymatous changes consisted of dilatation and proliferation of the ductular system, acinar dilatation and atrophy, and disruption of islets by intra-islet-ductular dilatation (Figs. 9, 10, and 11). In the animal killed one day after ligation, the pancreas showed increased mitotic activity of duct cells and mild dilatation of the lumen of ducts of all sizes. Many centroacinar cells appeared almost completely vacuolated, and some were disintegrating. This resulted in the formation of small cavi-

ties inside acini. The islets were quite normal in appearance, except that in approximately 30% of them a beta cell in mitosis was seen. In the two-day rabbit the ductular epithelium still showed marked proliferative activity and the cells were hypertrophic, with prominent cytoplasm and large, irregular, vesicular, closely packed nuclei. On the other hand, mitoses in the beta cells were not so numerous as in the one-day animal. Many ducts and acini showed mild dilatation of the lumen, and a few dilated ductules were occasionally seen inside islets. Seven days after ligation of the main pancreatic duct the proliferation of the ductular system reached its peak. The terminal ductules of the acini and the islets were considerably dilated, and many pancreatic islets were almost completely destroyed by this intraislet-ductular dilatation and concomitant proliferation (Fig. 11). These ducts were remarkable for the lack of material in their lumina, in contrast with those of previous stages, which did contain some material. Twenty-one days after duct ligation most acinar cells had disappeared. The pancreatic lobules consisted of islets, with more or less irregular contours, surrounded by masses of duct tissue, some with small lumina and others with dilated ones. There were many large duct cells with pale-staining cytoplasm, which contained one or two large vacuoles and an irregular lobated nucleus. Some ducts had dilated lumina, which, however, were always empty (Figs. 10, and 11). Fifty days after ligation the pancreas was characterized by disappearance of acinae, dilatation of ducts, and marked interlobar and intralobular fibrosis. It was also obvious that a large number of islets had disappeared. The remaining ones were composed of cords of islet cells, dispersed throughout the connective tissue, somewhat resembling the primary islets of the embryo. The cytology of the alpha, beta, and delta cells was practically unaltered in all of the duct-ligated animals.

Comment

The degree of diabetes and the type of lesions observed in the cortisone-treated groups were similar to that reported previously.[1] The failure to produce ductular hyperplasia in animals subjected to partial or complete starvation suggests that weight loss is not a primary etiological factor in the development of this lesion. Furthermore, treatment with vitamins, in addition to cortisone, did not inhibit the development of hyperplasia of the ductular system. It is also clear that alloxan diabetes did not bring about the production of the duct lesion, in spite of the fact that the duct epithelium showed marked hydropic change. It seems, therefore, that neither the disturbances in nutrition nor the diabetes itself, induced by cortisone, is responsible for this morphologic picture.

In unpublished observations, it was also found, contrary to previous findings in the rat,[22] that concomitant treatment with growth hormone did not prevent the weight loss that results from cortisone therapy in the rabbit. Furthermore, this combined therapy did not alter the degree of diabetes, nor did it prevent the development of the diffuse ductular hyperplasia in the pancreas.

The problem of infection is somewhat more complex. The lesions observed in the pancreas of animals treated with cortisone with and without antibiotics are morphologically identical. However, groups receiving antibiotics seemed to tolerate the cortisone better and survived longer than had previous groups which did not receive antibiotic therapy.[1] On the other hand, despite the antibiotic therapy, histologic evidence of chronic inflammation was present in various tissues of most treated animals. However, the pancreatic lesion is not that described in chronic pancreatitis of infections or of toxic origin.[23]

A comparison of the pancreas of duct-ligated rabbits with the cortisone-treated ones showed definite similarities. In both, there were marked hyperplasia and hypertrophy of the ductular system, and hyper-

plastic and dilated ducts were frequently found inside islets, causing irregularity in the shape of the islets. In addition, the two groups showed strands and cords of islet cells, which apparently arose by disruption of preformed islets through intraislet-duct dilatation. Also, focal areas of pancreatic necrosis, peripancreatic necrosis, and peripancreatic granulomatous inflammation were present in the duct-ligated and the cortisone-treated groups.

However, some distinguishing features became clearer with progression of the lesion. In the duct-ligated pancreas, the changes were uniformly distributed and of equal degree as the lesion progressed. There were dilatation of the larger ducts and almost complete disappearance of acinar tissue, so that the pancreas came to consist of some proliferating dilated ductular elements interspersed in fibrous tissue. Within this fibrous tissue were strands of alpha, beta, and delta cells, which usually did not show connections with the duct system. There was also a reduction of the total amount of islet tissue.

In the cortisone-treated pancreas, the morphologic alterations were present throughout the pancreas but were focal in character and at the same time unequal in degree. There was no dilatation of the large ducts. The acinar tissue was relatively normal except in focal areas, usually confined to a single lobule. In the areas with normal acinar tissue, there were marked proliferation and some dilatation of the intercalated ducts. The islets in these areas were often large and irregular and connected with proliferating duct epithelium. In focal areas, lobules of pancreatic tissue were seen which were almost identical with the duct-ligated pancreas of 7 or 21 days. Similar areas were also occasionally found in untreated rabbits. Another significant difference between the duct-ligated and the cortisone-treated pancreas was that in the latter the material present in the ducts was more aldehyde-fuchsin-positive and was often inspissated and laminated. This was in contrast to the pancreas of the duct-ligated animal, in which very little fluid was found within ducts after 48 hours.

Other workers have studied the pancreas of cortisone-treated rats,[24] guinea pigs,[25] rabbits, cats, dogs,[26] and monkeys,[27] but have not reported this type of proliferative lesion. This may be due to species differences or to differences in dosage and duration of therapy. On the other hand, pancreatitis and pancreatic fat necrosis have been stated to occur in rabbits after injection of 4 to 8 mg. daily of cortisone for 13 to 81 days. In the latter study, focal areas of acinar-cell degeneration were followed by atrophy and ductular proliferation and concretions were occasionally present in intralobular ductules. Inflammatory changes were mild, and fibrosis was present in older lesions. Hyperplasia of the islets of Langerhans was also stated to be often present. The blood amylase was elevated in these rabbits, and it was suggested that this was a form of pancreatitis which was related to the hyperlipemia induced by cortisone. It was considered to be analagous to human cases of essential hyperlipemia with recurrent pancreatitis.[28]

This explanation could apply to the lesions observed in our material. However, in spite of the differences between the lesion of cortisone-treated rabbits and that observed in duct-ligated rabbits, the similarities suggest that the cortisone lesion also may be obstructive in nature. The differences between the two types are thought to be due to the fact that in the cortisone-treated rabbit the obstruction is incomplete, and possibly intermittent in character. Furthermore, it is hypothesized that the obstruction may be situated anywhere from the level of the intralobular ducts up to the smallest intercalated ductules. This could account for the fact that a picture similar to that observed in a pancreas with the main duct ligated may be found in a single lobule of the cortisone-treated pancreas. The mechanism for this obstruction is not known, but it is considered that changes in viscos-

ity of the secretory material present in the ductules may be of importance. On the other hand, infection could not be ruled out as a possible factor. It is also still possible that high levels of adrenal steroids exert a direct stimulative effect on the pancreatic duct epithelium similar to the effect of other steroids on the breast or prostate.

Summary

An attempt is made to determine the pathogenesis of the diffuse ductular hyperplasia which was previously reported to occur in cortisone-treated rabbits. In the present study, it is found that definite lesions are observed as early as eight days, and that neither antibiotics nor vitamin supplements (A,B,C, or D) influence the morphologic pattern.

The pancreas from animals with alloxan diabetes, or from rabbits subjected to partial or complete starvation, does not present the ductular change associated with cortisone therapy. However, definite similarities are found between the morphology of the duct-ligated and that of the cortisone-treated pancreas. This similarity is taken to indicate that in the latter the lesion is also obstructive in nature, possibly caused by cortisone-induced changes in the viscosity of the secretory material. A possible role of infection, or of a direct response of ductular epithelium to cortisone, cannot be ruled out. The differences in the appearance of the duct-ligated and of the cortisone-treated pancreas are thought to be due to differences in the site of obstruction. In the latter, the obstruction may be either at or above the level of the intralobular ducts, whereas in the former the main pancreatic duct is obstructed. Furthermore, it is thought that the obstruction in the cortisone-treated animal is probably progressive, and possibly intermittent. This would account for the presence of lesions of varying degrees in a single section of the cortisone-treated pancreas, as compared with the uniformity of the appearance of the duct-ligated pancreas at any given stage.

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Bone Metabolism and Bone Resorption After Parathyroid Extract

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Accompanying the various morphologic transformations of bone matrix there are changes in the cells that suggest changes in their carbohydrate metabolism.* For example, at sites of new bone formation osteocytes and osteoblasts contain aggregates of glycogen. In the bone resorption following administration of large doses of parathyroid hormone, these intracellular stores of glycogen are diminished.† To bridge the relationship between such histochemical observations and cellular metabolism, we have studied the respiration and anaerobic glycolysis of metaphyseal bone.[5] In comparison with most other tissues, normal bone had an extremely low rate of carbohydrate metabolism. There was evidence, however, for the operation of the Krebs cycle. Following parathyroid administration, anaerobic glycolysis was unaffected, but the oxygen consumption of the bone cells was markedly depressed. This decrease in respiration was related to a diminished dehydrogenase activity. From these observations and other indirect evidence, a theoretical explanation for the resorption of bone is offered.

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* References 1, 2.

† References 3, 4.

Material and Method

Preparation of the Tissue

Metaphyseal bone was obtained from a group of 95 normal weanling albino rabbits, approximately 6 weeks of age, and from a similar group of 61 animals which had received 1000 units of parathyroid U. S. P.‡ in divided doses 42 and 28 hours prior to the time of analysis. The animals were killed by a blow on the head, and the distal end of the femur and the proximal end of the tibia were excised. The bony epiphyses, together with the epiphyseal cartilage, were removed. The diaphyseal portions were split longitudinally and cleaned of marrow. Thin slices of metaphyseal bone were then cut freehand with a razor blade. Representative sections were frozen-dried for histological and histochemical investigation. These tissues were embedded in paraffin, cut, without decalcification, at 6μ, and deparaffinized in xylene. The sections were denatured in absolute alcohol, before staining. Carbohydrate-protein complexes were identified, using the periodic acid-leucofuchsin method.[6] Glycogen was demonstrated as red-staining material which could be dissolved by pretreatment of sections with β-amylase at pH 6. Mitochondria were stained, after chromation in Regaud's solution, with acid aniline fuchsin. A number of sections were stained with hematoxylin and eosin.

Determination of Respiration and Anaerobic Glycolysis

Respiration and anaerobic glycolysis of the metaphyseal bone slices were studied by the standard manometric methods.[7] The suspending medium for the endogenous aerobic determinations consisted of 2.8 ml. of Krebs-Henseleit calcium-free phosphate buffer, pH 7.4, with 0.2 ml. of 2 N KOH in the center well of the flask. All added substrates, except dextrose, were present in a final concentration of 0.018 M. These included the sodium salts of pyruvate, citrate, succinate, fumarate, and α-ketoglutarate. The substrates were added directly to the flasks at the start of the experiment in 0.5 ml. aliquots, replacing an equal amount of buffer. Dextrose was used in

‡ Eli Lilly & Company contributed a supply of the parathyroid extract.

final concentration of 0.039 M. The effect upon respiration of methylene blue (7×10^{-5}M) and sodium malonate (0.019 M) was also investigated. These compounds were added to the flasks from the side-arm after 30 minutes. The suspending medium for the study of endogenous anaerobic glycolysis consisted of 3.0 ml. of Krebs-Ringer bicarbonate buffer. In a number of determinations dextrose was present in a 0.039 M concentration.

Approximately 250 mg. of sliced bone was placed in each flask. The manometers for the respiratory studies were gassed for 10 minutes with 100% oxygen, while those for the anaerobic investigations were gassed with a mixture of 95% nitrogen and 5% carbon dioxide. The flasks were placed in a constant temperature bath maintained at 38 C, and, after an equilibration period of 10 minutes, the manometers were read at 15-minute intervals for an hour. Upon completion of the experiment, the bone slices were either dried at 100 C for 12 hours and reweighed or were extracted, using the method of Schneider,[8] and analyzed for deoxyribonucleic acid content by the method of Dounce.[9] The rate of respiration was expressed as microliters of oxygen utilized per hour per milligram of dry tissue (QO_2) or per milligram of deoxyribonucleic acid (QO_2 [DNA]). Anaerobic glycolysis was expressed only as microliters of CO_2 produced per hour per milligram of deoxyribonucleic acid ($Q_{CO_2}^{N_2}$ [DNA]) Since the metabolically inert matrix of bone contributes the largest part of the tissue mass, relation of the rates to the DNA content of the tissue was regarded as a more reliable index of cellular metabolism. It is claimed that the quantity of DNA per nucleus is approximately the same in most tissues.[10]

Reduction of Triphenyltetrazolium Chloride

The reduction of triphenyltetrazolium chloride (TTC) by metaphyseal bone slices and bone marrow was determined, using a modification of the method of Kun and Abood.[11] Approximately 250 mg. of tissue was added to a 15 ml. Warburg flask containing 1.5 ml. of 0.1 M phosphate buffer (pH 7.4), 0.5 ml. of 0.2 M sodium succinate, and 1.0 ml. of 0.1% TTC solution. The standard contained an equal amount of tissue plus 2.95 ml. of buffer and 0.05 ml. of TTC, but no succinate. The blank consisted of 250 mg. of tissue, 2.5 ml. of buffer, and 0.5 ml. of 0.2 M sodium succinate. A small number of determinations were also made without the addition of a sodium succinate substrate to the tissue samples.

After the tissue was added, the flasks were gassed for 5 minutes with 95% nitrogen and 5% carbon dioxide and were then shaken at 38 C for 30 minutes. Immediately thereafter a minute amount of sodium hydrosulfite was added to the standard to develop full color. The various tissue samples were then extracted by vigorous shaking with acetone for 20 minutes. The solutions were centrifuged, and the supernatant fractions were withdrawn for the determination of optical density at 475 mμ. The deoxyribonucleic acid content of the tissue was determined, and the results were expressed as micrograms of triphenyltetrazolium chloride reduced in 35 minutes per milligram of DNA.

Supravital Staining with Triphenyltetrazolium Chloride

Four normal weanling rabbits and four which had been pretreated with parathyroid U. S. P. (two 500-unit doses over a 44-hour period) were perfused through the descending aorta with 500 cc. of 0.1% triphenyltetrazolium chloride. The animals were allowed to remain at room temperature for 30 minutes, after which the femur and tibia were removed, split longitudinally, and examined grossly.

Results

Respiration and Anaerobic Glycolysis of Metaphyseal Bone Slices

The manometric results are summarized in Tables 1 to 3. Animals treated with parathyroid showed a mean QO_2 of -0.45, as compared with the control values of -0.63. When respiration was related to the deoxyribonucleic acid concentration, the corresponding QO_2(DNA) values were -33 and -57, respectively (Table 1). These differences were highly significant ($P<0.01$) and represent a depression of respiration in the hormone-treated group of approximately 40%.

In an attempt to determine points of enzymatic inhibition, the effect on respiration of various added substrates was studied. Dextrose, pyruvate, citrate, α-ketoglutarate, and fumarate did not appear to affect the rate of respiration significantly in either group (Table 2). The addition of methylene blue was also ineffective. Sodium succinate, however, produced approximately a twofold increase in the oxygen uptake of bone slices from both groups but did not elevate the respiration in the treated animals to the normal level (Table 2). The stimu-

TABLE 1.—*Endogenous Respiration of Metaphyseal Bone from Normal and Parathyroid-Treated Weanling Rabbits*

	No. of Experiments	Mean QO_2* (±S.D.)	No. of Experiments	Mean QO_2 (DNA)† (±S.D.)
Control	67	−0.63±0.09	18	−57±4
Treated	32	−0.45±0.10	19	−33±11
		$P<0.01$		$P<0.01$

* Expressed as microliters per hour per milligram dry weight.
† Expressed as microliters per hour per milligram deoxyribonucleic acid (DNA).

TABLE 2.—*Effect of Added Substrate Upon Respiration of Metaphyseal Bone from Normal and Parathyroid-Treated Weanling Rabbits*

Substrate	No. of Experiments	Mean QO_2 (DNA) Normal Animals	No. of Experiments	Mean QO_2 (DNA) Treated Animals
None	67	− 57.0	32	−33.0
Dextrose	8	− 57.3	12	−26.3
Pyruvate	4	− 56.8	6	−38.6
Citrate	8	− 41.1	2	−30.0
α-Ketoglutarate	2	− 54.2	4	−37.4
Succinate	2	−104.7	2	−70.7
Fumarate	4	− 58.4	3	−34.0

TABLE 3.—*Anaerobic Glycolysis of Metaphyseal Bone from Normal and Parathyroid-Treated Weanling Rabbits*

	No. of Experiments	Mean $Q_G^{N_2}$*(DNA)* (±S.D.)
Control	18	+33.2±15.4
		$P<0.05$
Treated	19	+27.8± 9.8

*Expressed as microliters per hour per milligram deoxyribonucleic acid.

lating effect of succinate was inhibited by the subsequent addition of sodium malonate to the suspending medium.

The rates of endogenous anaerobic glycolysis in the control and the treated animals did not differ significantly, averaging +33.2 in the former and +27.8 in the latter group (Table 3). This finding minimizes the possibility that the changes observed in respiration were the result of a nonspecific reaction of the cells to massive doses of the hormone. Upon addition of dextrose to the suspending medium, the rate of anaerobic metabolism in the bone slices from both series was increased, being approximately doubled in the normal rabbits (+67.6±17) and trebled (+85.2±13) in the hormone-treated animals.

Dehydrogenase Activity of Metaphyseal Bone Slices

Dehydrogenase activity was further studied by measuring the reduction of triphenyltetrazolium chloride. When no substrate was added, only small amounts of TTC were reduced by metaphyseal bone slices from normal rabbits (Table 4). When

TABLE 4.—*Reduction of Triphenyltetrazolium Chloride by Metaphyseal Bone Slices from Normal and Parathyroid-Treated Rabbits*

	No. of Experiments	Substrate	Amt. TTC Reduced*
Control	10	Succinate	75.3±6.8 γ†
	3	None	12
Treated	10	Succinate	30.8±3.6 γ†
	1	None	9.6

* Expressed as micrograms of triphenyltetrazolium chloride reduced per 35 minutes per milligram of deoxyribonucleic acid—means and standard deviations.
† The differences between these values in control and treated animals were highly significant; $P<0.01$.

sodium succinate was added, an average of 75.3γ of TTC was reduced in 35 minutes per milligram of DNA. This increase was ascribed largely to the activity of succinic dehydrogenase. The succinic dehydrogenase activity of the cells from animals treated with parathyroid was approximately 60% less (30.8γ TTC/35 min/mg. DNA). For marrow the values were even lower, and no significant difference was found between the two groups (Table 5). This would seem to exclude any important contribution to the values obtained from the bone slices by hematopoietic elements in the metaphysis.

TABLE 5.—*Reduction of Triphenyltetrazolium Chloride by Bone Marrow from Normal and Parathyroid-Treated Rabbits*

	No. of Experiments	Substrate	Amt. TTC Reduced*
Control	10	Succinate	23±16 γ
Treated	9	Succinate	26±16 γ

* Expressed as micrograms of triphenyltetrazolium chloride reduced per 35 minutes per milligram deoxyribonucleic acid—means and standard deviations.

The diminished dehydrogenase activity of bone from the hormone-treated animals could also be demonstrated qualitatively in the gross specimens from the rabbits perfused with triphenyltetrazolium chloride. In the control animals the entire metaphysis was stained red, due to the formation of an insoluble formazan; but in animals receiving parathyroid only small amounts of formazan were precipitated.

Histochemical and Histological Observations

In addition to the well-described morphologic transformations that occur in bone under the influence of parathyroid,§ certain other histological and histochemical alterations have been noted in this study which may be related to the changes found in carbohydrate metabolism. In young normal animals the connective tissue cells contiguous to bone and many of the osteoblasts and osteocytes contained aggregates of glycogen (Fig. 1). Following administration of parathyroid the intracellular glycogen aggregates were significantly reduced (Fig. 2*A*). Bone matrix, which ordinarily stains lightly with the periodic acid-leucofuchsin reagents, was deeply stained in areas of active resorption, and fragments of this bone were dispersed throughout the diaphyseal part (Fig. 2). The description coincides essentially with that given for the rat.‖

In preparations stained for mitochondria, these inclusions were seen to be only sparsely distributed in osteoblasts and only occasionally demonstrated in osteocytes (Fig. 3). Many mitochondria were found within the osteoclasts. It was not possible to make any qualitative or quantitative distinctions between the treated and the untreated animals.

Comment

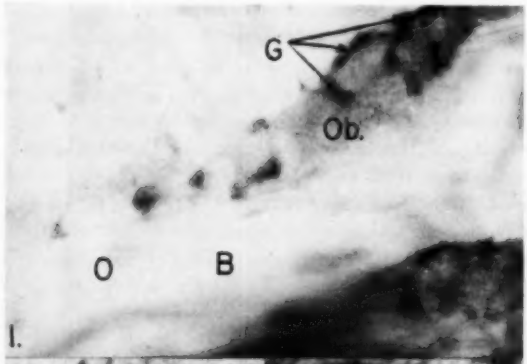
In studies on the metabolism of bone, attention has been directed largely to the anaerobic phase of carbohydrate degradation, particularly to the relationship between the phosphorylases and phosphatases and the process of calcification. It has been assumed that the respiration of bone cells is comparatively low, and the oxidation of carbohydrates through the Krebs cycle has received little attention. Recently, however, Dixon and Perkins¶ have demonstrated citrogenase, aconitase, and isocitric dehydrogenase in bone, and the presence of cytochrome oxidase has also been claimed.[16] Evidence for the operation of the Krebs cycle in bone is further strengthened by our observations on succinic dehydrogenase activity, including the stimulation of respiration by succinate and its inhibition by malonate.

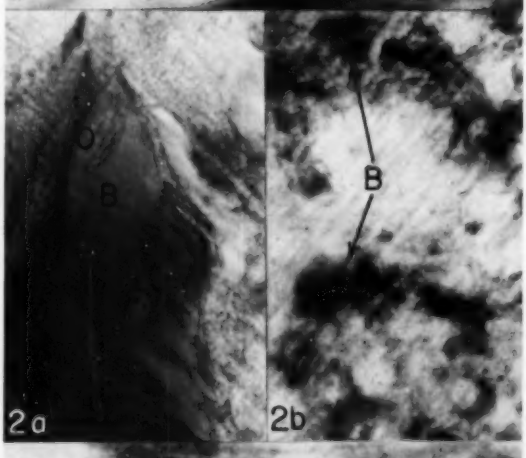
By comparison with that for other tissues, the rate of carbohydrate metabolism of metaphyseal bone appears to be relatively low (Table 6). For example, the oxygen uptake is about one-twenty-fifth that of

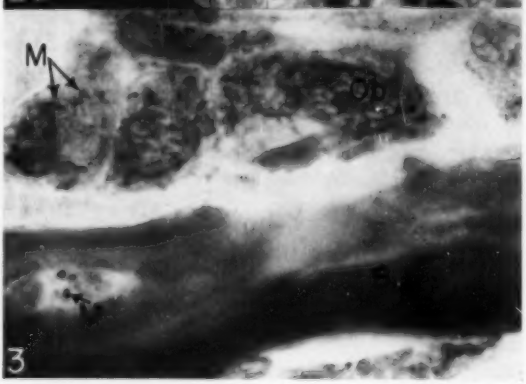
§ References 3, 12.

‖References 1, 13.

¶ References 14, 15.







Sections through metaphysis of tibia from weanling rabbit. All tissues were prepared by freezing-drying without decalcification and were cut at approximately 6μ. Figures 1 and 2 were stained for carbohydrate-containing substances with the periodic-acid-leucofuchsin reagents. Figure 3 was stained for mitochondria with aniline acid fuchsin after chromation in Regaud's solution. *B*, bone; *G*, glycogen; *M*, mitochondria; *O*, osteocyte; *Ob*, osteoblast.

Fig. 1.—Bone from untreated (control) rabbit. The osteoblasts bordering the spicule contain dark-staining aggregates of glycogen. The bone is barely stained by the reagents; reduced to 80% of original approximate magnification of × 850.

Fig. 2.—Bone from animal which received 1000 units of parathyroid U. S. P. in 44 hours; reduced to 80% of original approximate magnification × 850. (*a*) The contiguous cells are free of glycogen. There has been a change in the bone matrix, as shown by its intense staining reaction. (*b*) The dark masses are heavily stained remnants of disaggregated bone.

Fig. 3.—Distribution of mitochondria in cells of bone from normal weanling; reduced to 80% of original approximate magnification × 1500.

liver and kidney per unit of deoxyribonucleic acid. Comparable differences in the aconitase, citrogenase, and isocitric dehydrogenase levels between these tissues have also been reported.[14] If the respiratory enzymes are concentrated in the mitochondria, then the comparatively low oxygen consumption of the bone slices may be consistent with the relative paucity of mitochondria observed in the osteoblasts and osteocytes.

Following the administration of parathyroid, respiration in the bone slices was markedly reduced, despite the addition of

TABLE 6.—*Respiration and Anaerobic Glycolysis of Various Animal Tissues**

Tissue	Q_{O_2} (DNA)	$Q_O^{N_2}$ (DNA)
Kidney	−1400	+200
Liver	−1320	+275
Costal cartilage	−273	+467
Spleen	−240	+160
Bone	−57	+33

* Calculations based upon data taken from the literature.[15] Values expressed as microliters per hour per milligram of deoxyribonucleic acid. Dry weight/wet weight ratio used for bone was 40%; for soft tissues, 25%. DNA/DNAP ratio, 9.89.[14]

dextrose, pyruvate, and some of the di- and tricarboxylic acid intermediates of the Krebs cycle. This may be due in part to a depressing effect upon certain of the dehydrogenating enzymes. Diminished succinic dehydrogenase activity was demonstrated in our experiments. Conceivably, the resultant decrease in the rate of oxidation of intermediary metabolites could lead to their accumulation in the cells and extracellular environment. These more intimate details of carbohydrate metabolism were not studied. However, they may be of great importance for the general problem of bone resorption. For example, it has been shown that many of the di- and tricarboxylic acid intermediates of the Krebs cycle can react in a unique way with calcium and magnesium, forming relatively un-ionized soluble complexes or chelates with the phosphates and carbonates at physiological pH.# Then, if these intermediates accumulate in the neighborhood of bone, they could compete for cations with the mucoproteins and other negatively charged colloids composing the bone matrix.[22] It may be postulated that the calcium salts would thereby be brought into solution. Since the aggregate state of the matrix is related to the binding effects of these cations, simultaneously with the loss of minerals there would be a disaggregation of the organic components. This does not exclude the possibility that proteolytic enzymes secreted by osteoclasts and other connective tissue cells could also contribute to the disaggregation.

In the case of bone, where such important substances in cellular metabolism as citrate, phosphate, and carbonate are also integral parts of the extracellular structure, relations among form, energy, and metabolism are implicit. Then, in normal and pathologic states, the apposition and resorption of bone may be related to variations in the metabolism of the contiguous cells.

References 17-21.

Summary

Some aspects of carbohydrate metabolism were studied in metaphyseal bone slices from normal and parathyroid-treated rabbits. The respiration of normal bone was found to be considerably lower than that reported for most other tissues. Whereas anaerobic glycolysis was unaffected following the administration of parathyroid (Parathormone), the oxygen consumption of the bone slices was diminished. This finding was attributed in part to a depression of the succinic dehydrogenase system. These metabolic findings were associated with a decrease in the intracellular glycogen in the osteoblasts and osteocytes and a disaggregation of the glycoprotein ground substance. A theory of bone resorption is proposed that considers the potential solubilizing effect on bone of some of the intermediate products of cell metabolism.

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Brain Abscess Due to Hormodendrum Species

REPORT OF THIRD CASE

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Most of the fungi that are ordinarily recognized as pathogenic for man, particularly those that cause the "systemic" mycoses, have been demonstrated to produce infection of the central nervous system. With the possible exception of cryptococcal meningitis, the majority of such cases represent involvement of the central nervous system as a late or terminal complication of widespread infection of other parts of the body. Because of the relative rarity of mycotic brain abscesses, a case of mycotic brain abscess, observed locally and followed to necropsy, that did not show evidence of infection in any other organ was considered rather unusual. An even more remarkable feature was the identity of the fungus cultured from the lesion. The striking similarity of this case to two previously reported cases with respect to clinical course of the patient, reaction of the tissues to the organism, and the identity of the fungus appeared to us to make it worthy of a brief report.

Report of a Case

Clinical History.

This Negro woman, a lifelong inhabitant of New Orleans, was seen at this hospital many times in the 19 years prior to death because of illnesses apparently unrelated to the lesion that eventually caused her death.

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She was first seen at the age of 19 years because of a laceration of the scalp. At the age of 21 she was admitted because of precordial discomfort and dyspnea, of two weeks' duration. Physical examination of the heart and lungs was negative. The teeth were noted to be extremely carious, and the Wassermann reaction was strongly positive. No specific diagnosis was recorded.

The patient was again admitted at the age of 24 for malaria, and was treated successfully with quinine; in the same year she was also treated for subungual abscesses of the fingers.

At 26 years of age the patient was again admitted because of chills and chest and back pain. She had developed a sore on the left labium minus two months previously, and one month following intercourse. Physical examination disclosed only carious teeth, purulent vaginal discharge, and a large ulcer of the left labium minus. The urine contained pus cells, and the blood Wassermann reaction was again strongly positive. The cerebrospinal fluid Wassermann reaction was negative. She received methenamine (Urotropin), several injections of neoarsphenamine, and other antisyphilitic therapy in the clinic, but there is no record of her having completed the course of treatment.

Between the ages of 29 and 33 years she was seen three times because of superficial traumatic injuries to the scalp.

The history of the terminal illness began at the age of 37, when the patient awoke one morning and found she was almost completely paralyzed on the right side. She was seen in the clinic for these symptoms, but no definite diagnosis was established and she improved considerably without any treatment. However, about one month after the onset she had a convulsion, beginning in the right foot and progressing upward to involve the right arm, and a few hours later she was admitted to the hospital.

At the time of admission her temperature was 98.6 F and her blood pressure 120 systolic and 70 diastolic. She was in no acute distress but had an obvious right hemiplegia. The pertinent abnormalities were elicited on neurological examination and were limited to the right side. They consisted of (1) marked weakness of the muscles

of the arm and leg; (2) hyperactive deep tendon reflexes; (3) diminution of the corneal reflex; (4) weakness of the facial muscles, and (5) partial anesthesia of the palate. The blood hemoglobin was 13 gm. per 100 ml.; the white blood cell count, 8000 per cubic millimeter, and the differential count, normal.

X-ray films of the skull disclosed no abnormality. Electroencephalograms were reported as showing an "irregular, extremely slow wave focus, left anterior and presumptive left temporal. Very strongly suggestive of symptomatic epilepsy and presumptive evidence of brain damage."

The patient gradually regained strength without specific therapy and was discharged much improved. However, within eight days her hemiplegia became much worse and she had another right-sided seizure, and was readmitted. Physical and laboratory findings were the same as before. X-rays of the skull were again negative. A pneumoencephalogram was interpreted as showing a "space-occupying lesion in the left parietal region."

Soon thereafter, or about three months after the onset of symptoms, a craniotomy was performed. In the left parietal lobe a firm mass was located and easily removed by enucleating it with the finger. In its removal the mass fell to the floor and ruptured. A culture was taken from the surface of the puddle and the remainder fixed in formalin.

The culture was reported as showing no growth in 48 hours and was then discarded. After fixation, the specimen was a shell of grayish-yellow tissue 1 to 5 mm. thick, forming a cyst-like cavity 4 cm. in diameter. This cavity contained gray, pasty, opaque material, and the interior surface of the wall was quite shaggy.

Cyst Wall.

Histologically, this wall was composed of dense collagenous tissue toward the inner surface, becoming loose and vascular nearer the outside and merging with glial tissue (Fig. 1). Within this fibrous wall were numerous small abscess pockets filled with polymorphonuclear leucocytes and necrotic debris, with an occasional multinucleated giant cell about the edge. Both in giant cells and lying free among the debris were numerous brown, segmented, filamentous, branching structures, each segment measuring approximately 5μ in diameter and 15μ in length (Fig. 2). The terminal segment was often club-shaped. The diagnosis reported at that time was "brain abscess due to a fungus, probably Candida albicans."

Postoperative Course.

The patient's paralysis improved for a time following operation, and she was discharged. Two months later she was much worse, having slurred speech, left-sided headaches, fever, and a fluctuant mass at the site of the craniotomy. This mass yielded pus on aspiration and was opened widely. Culture of the pus was negative for bacteria, but a fungus was observed both by direct examination and after culture, which will be described later.

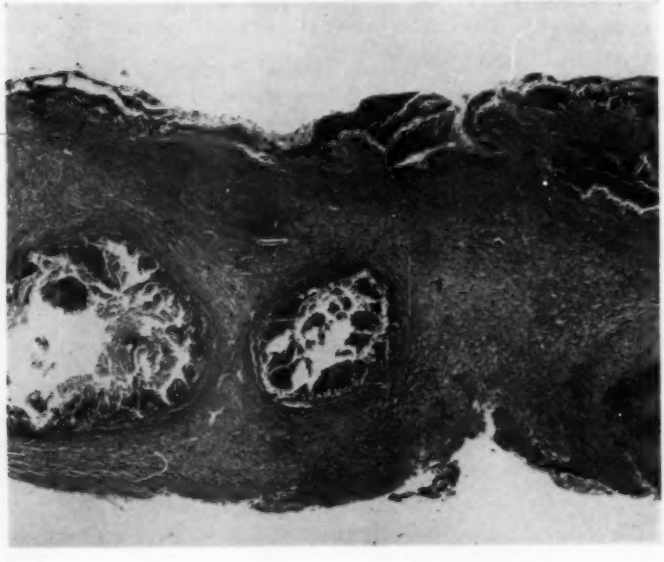

Fig. 1.—Wall of brain abscess due to Hormodendrum species, surgical specimen. The upper edge shows the shaggy layer of necrotic material on the inside of the wall. The major portion of the wall is composed of collagenous and glial tissues and contains many smaller abscess cavities, two of which are shown here. Hematoxylin and eosin stain; reduced to 40% of mag. × 17.

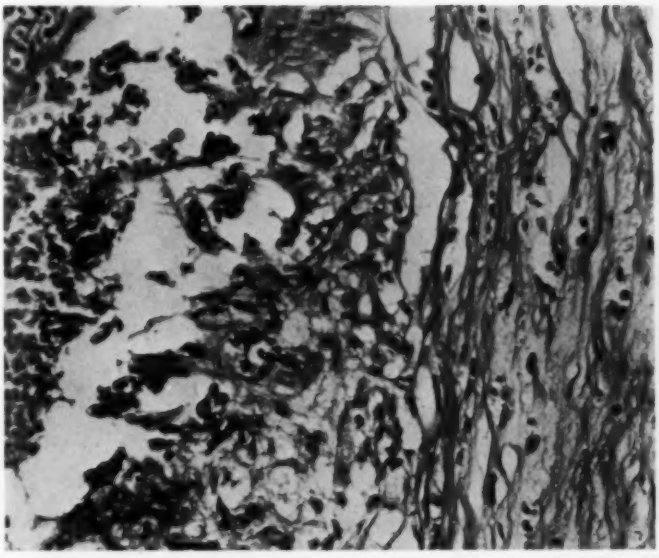

Fig. 2.—Higher magnification of the tissue at the edge of one of the small abscesses seen in Figure 1, showing the septate branching hyphae. Notice the variation in size and shape of the hyphae, which are made very prominent by their dark-brown color. Hematoxylin and eosin stain; reduced to 40% of mag. ×625.

Soon thereafter she was readmitted with a nearly complete right hemiplegia and a large mass beneath the scalp, from which yellow purulent material drained through two openings. Another craniotomy was performed, and a large, poorly defined abscess cavity in the parietal lobe was incised and evacuated. Examination of this material yielded the same fungus as before.

Following this operation the patient became progressively worse, finally lapsing into a vegetative state. The temperature varied from normal to 101 F, occasionally rising to 102 F. Solutions of bis-trimethylenediaminocupric sulfate were instilled into the abscess cavity but produced no significant effect. The patient died seven and a half months after the onset of symptoms.

Summary of Pertinent Necropsy Findings.

The body was emaciated, and there were decubitus ulcers on the right buttock and shoulder. There was a soft fluctuant swelling of the scalp, 4 cm. in diameter, in the anterior left parietal region, and beside it was a defect in the scalp 3×1 cm., front which was escaping a pink gelatinous material. Approximately three-fourths of the teeth were missing or were carious stumps. Otherwise the eyes, ears, nose, and mouth appeared normal. The peritoneal, pleural, and pericardial cavities showed no significant abnormalities. The heart weighed 220 gm. and was grossly normal. The right lung weighed 400 gm., and the left, 360 gm. There were a few poorly defined dark-red areas of consolidation in the lower lobe of both lungs, but, despite a careful search, no nodules suggesting old or recent granulomatous lesions could be found in the lungs, bronchi, or hilar nodes. The liver weighed 1100 gm. and was grossly normal. The spleen weighed 80 gm.; the pulp was soft and mushy. The entire alimentary tract, from pharynx to rectum, was normal. The kidneys weighed 110 and 120 gm. right and left respectively, and were normal. The pancreas, adrenals, and thyroid were all normal. The uterus contained several leiomyomata, but otherwise the internal genitalia were normal.

The outstanding findings were limited to the brain. The fluctuant swelling beneath the scalp was found to be brain tissue herniated through a defect 6 cm. square in the skull. The scalp and dura were firmly adherent to the edges of the defect. After the calvaria was removed and material for culture taken from the brain, the brain was injected with and suspended in formalin for fixation before sectioning.

The hypophysis and its bony surroundings appeared normal. The petrous bone, the middle ear, and the ethmoid, sphenoid, and frontal sinuses were normal. The tympanic membranes were intact and were not scarred.

Upon further examination of the brain after fixation, the anterior half of the left parietal lobe appeared as a shaggy, friable mass with few recognizable structures remaining. In the center of the portion that had been extruded through the defect in the skull was an irregular opening, 2 cm. in diameter, in which was much creamy, greenish-yellow pus. The adjacent meningeal surfaces of the parietal and frontal lobes were discolored with mottled orange and yellow areas,

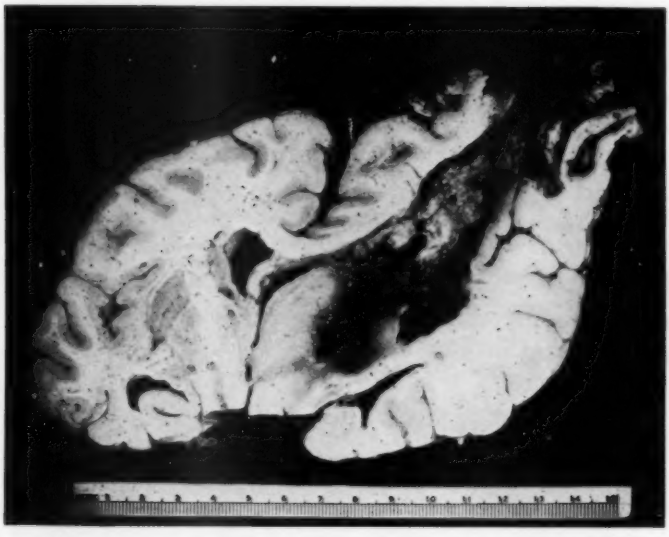

Fig. 3.—Coronal section of brain showing the large irregular abscess cavity in the left hemisphere with channel communicating to outside. The greatly distorted surface of the left hemisphere represents the portion that was being extruded through the cranial defect.

but there was no exudate and the remaining meninges appeared normal.

Upon coronal section of the brain (Fig. 3) all midline structures were seen to be displaced to the right. The right ventricle was dilated and the left ventricle reduced to a slit-like space. Replacing the left centrum ovale, left lenticular nucleus, and lateral part of the thalamus was a cavity filled with thick, creamy material, similar to that seen externally and rimmed with a layer of red- and brown-mottled brain tissue 0.5 to 1.0 cm. thick. This cavity communicated with the opening seen on the surface by way of a broad tract. Extending posteriorly into the occipital lobe were several isolated smaller abscesses, from 1 to 2 cm. in diameter, with sharply delineated borders of yellow tissue. At the level of the splenium of the corpus callosum was an orange and yellow dense fibrous scar, 2×2×5 cm., extending outward to the surface of the hemisphere, presumably representing the site of the brain abscess removed at the first craniotomy. The large abscess also communicated with the left lateral ventricle, and some exudate was found within all the ventricles.

The right hemisphere was distorted, as described above, but contained no lesions. The cerebellum, pons, and medulla were neither distorted nor involved by any lesions.

Summary of Histologic Description.

In the lungs were a few scattered areas of edema and a recent infarct, but no abscesses or granulomatous lesions of any kind were seen. The remaining organs, aside from the brain, were all essentially normal. A careful search was made for miliary focal lesions that might have been related to the lesions in the brain; none was found.

Sections of the brain were examined both in hematoxylin-eosin and periodic acid-Schiff stains. A slight degree of fibrosis and lymphocytic infiltration were present in the pia-arachnoid; no fungi were found. Within the substance of the brain were numerous irregular abscesses, some with a fibrous lining similar to the original surgical specimen; others consisted of a narrow ring of necrotic brain tissue filled with debris, foamy mononuclear cells and a few giant cells (Fig. 4). Just inside the zone of recent necrosis were found scattered fungal filaments identical in appearance with those seen in the surgical specimen (Figs. 5 and 6).

Anatomic Diagnoses

The final anatomic diagnoses were (1) brain abscess due to Hormodendrum species; (2) infarct of right lower lobe of lung, and (3) leiomyomata of the uterus.

Morphology and Cultural Characteristics of the Organism

Wet preparations of (1) the fixed material in the original abscess, (2) material obtained from the draining sinus of the pa-

tient during life, and (3) material obtained from the brain at necropsy contained fungal elements consisting of hyphae, straight and curved, branching and septate, with segments 20μ to 30μ long and 2μ to 10μ in diameter. The hyphae contained finely granular protoplasm of yellow-brown color and refractive droplets.

The initial attempt to recover pathogens, using material aspirated from the craniotomy site a few days following the first operation, gave negative results. However, a dark-brown mold was recovered repeatedly on artificial media inoculated with purulent material collected from the fluctuant mass that appeared at the original operation site two months later, and from brain tissue obtained by biopsy and at necropsy. Identical results were obtained by two additional laboratories independently from the same specimens.

Gross Morphology in Culture.—Growth of the fungus was noticeable as dark-brown to black felt-like colonies within six days at room temperature on Sabouraud's and rabbit blood agar media, the most luxuriant

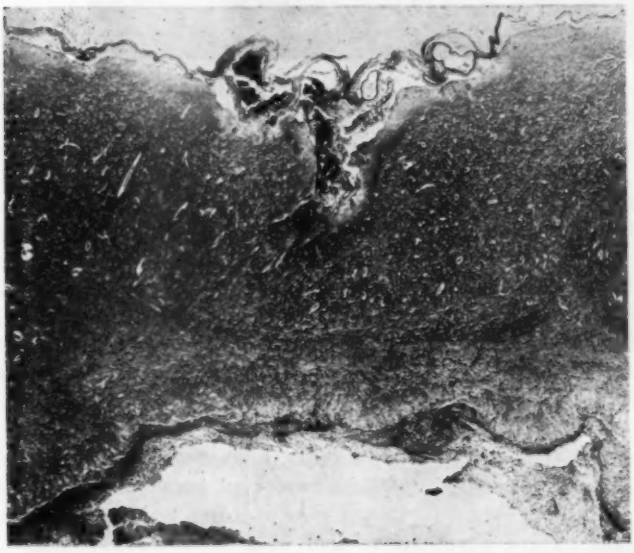

Fig. 4.—Left cerebral cortex with meninges at top and a portion of one of the extensive abscess cavities at the bottom of the photograph. The wall of the abscess is formed only by a zone of necrotic brain tissue and inflammatory exudate. Hematoxylin and eosin stain; reduced to 40% of mag. × 25.

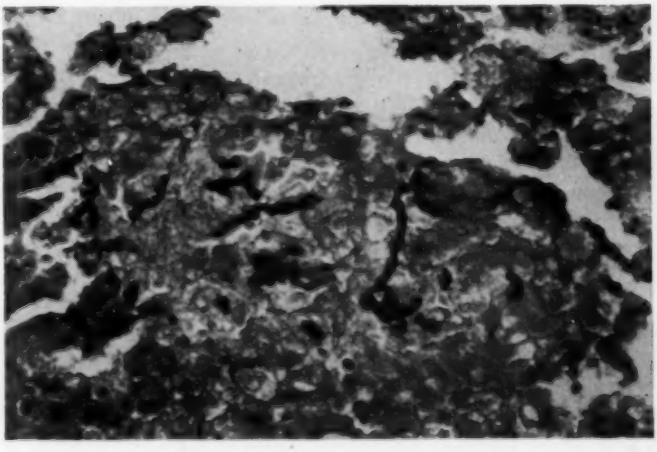

Fig. 5.—Hyphae of Hormodendrum species among necrotic debris in abscess from necropsy specimen. Hyphae are much less numerous as compared with the size of the lesion than in the original surgical specimen. Periodic acid-Schiff stain; reduced to 2/3 of mag. × 625.

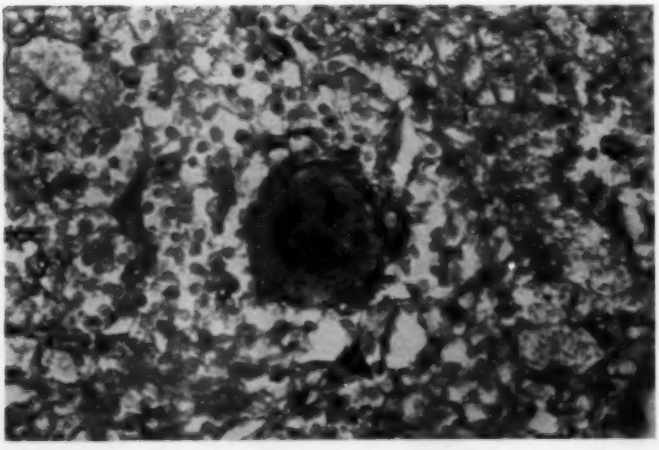

Fig. 6.—Hyphae of Hormodendrum species in giant cell located in wall of abscess from necropsy specimen. Periodic acid-Schiff stain; reduced to 2/3 of mag. ×625.

growth taking place on the Sabouraud medium.

Growth was detectable within eight days on the Sabouraud and blood agar media at incubator temperature, but again the fungus grew more luxuriantly on the Sabouraud medium.

The fungus, when grown on Sabouraud's medium at room temperature, after the second week, resembled flattened discs measuring about 5 cm. in diameter. The center of the culture was raised about 12 mm. above the medium. The culture gently sloped radially; the aerial mycelium was abundant, forming a dark-brown to black felt-like network.

On Czapeck's medium the colonies were not well developed; the mycelium was mostly submerged with only shallow layers of aerial mycelium, which were brown to black in color.

Microscopic Morphology in Culture.—The microscopic characteristics were more pronounced after the first week of growth on Sabouraud's medium at room temperature. The vegetating hyphae were straight or curved, branching and septate, with articles 10μ to 30μ long by 2μ to 10μ in diameter. The cell walls were thick and dark-brown; the protoplasm was yellow-brown, finely granular, and contained refractive droplets.

The sporulation was of the Hormodendrum type. The fertile branches were erect or ascending with the terminal cell, or conidiophore sometimes darker, having at the tip several tiny, truncate, conical structures, to which the spores were attached. The conidia were borne in chains that occasionally branched. The conidia were unicellular, ovoid, and elongated. They measured 3μ to 8μ by 1μ to 6μ, were dark-green to brown in color, and had smooth, thick, dark walls, and poorly developed disjunctors.

Biological Characteristics and Reactions.—This fungus grew best on an acid medium. The hydrogen ion concentration of the medium used in the cultural studies of this fungus ranged between pH4 and pH7. Its optimum temperature has not been precisely determined, but this fungus has been shown to grow well at 37 C and at room temperature (25 C).

The fungus stained with all routine bacteriological stains. Structural details were more evident when stained with lactophenol-cotton blue (aniline blue W. S.) and examined in wet preparation.

The fungus did not coagulate or peptonize litmus milk. Indol was not produced in Dunham's peptone water; the medium became dark brown on prolonged incubation. Sugars were not fermented.

Results of Animal Inoculation

A sample of pus taken from the brain at necropsy was diluted with equal parts of saline; penicillin and streptomycin were added, and 0.1 ml. of this mixture was injected into each of three mice intraperitoneally, three mice subcutaneously, and two mice intravenously. The mice inoculated intravenously died within a few minutes. Those inoculated intraperitoneally and subcutaneously survived without apparent effect for four weeks, at which time no lesions were found in any organs.

A 12-day culture of the fungus grown on Sabouraud's agar at room temperature was ground and suspended in 10 ml. of saline. Penicillin and streptomycin were added, and 0.1 ml. of this suspension was injected intracerebrally into each of three white mice. Eleven days later two of these mice were dead and the third quite ill. A fungus identical with that obtained from the brain of the patient was found in wet preparations, cultures, and sections from the brains of all three mice. Histologic examination disclosed it to be growing throughout the brain, with varying degrees of inflammatory reaction and necrosis. A distinct, well-demarcated abscess was not formed. No lesions were found in the other organs.

Material from the brain of the one mouse that was killed was ground and treated with penicillin and streptomycin, and 0.5 cc. was injected intraperitoneally into each of three mice and 0.03 cc. intracerebrally into three mice. All of these animals remained healthy. They were killed three months after inoculation; no lesions of any kind were found.

Five guinea pigs were each injected intraperitoneally with 0.5 ml. of the diluted pus. All of these died in from one week to six weeks following inoculation; no lesions associated with a fungus were found.

One guinea pig was injected subcutaneously with 0.5 ml. of the pus. Three weeks later the animal was killed, and at necropsy a small caseous nodule was found at the site of inoculation. There were no gross or microscopic lesions in any of the viscera. The subcutaneous nodule was cultured, and a fungus identical with that obtained from the patient grew on Sabouraud's agar. In the subcutaneous tissue was an aggregate of polymorphonuclear leucocytes, necrotic debris, and segmented brown hyphae, surrounded (Fig. 7) by a wall of fibrous tissue. The organisms and the tissue reaction both appeared similar to those seen in the original brain abscess removed from the patient.

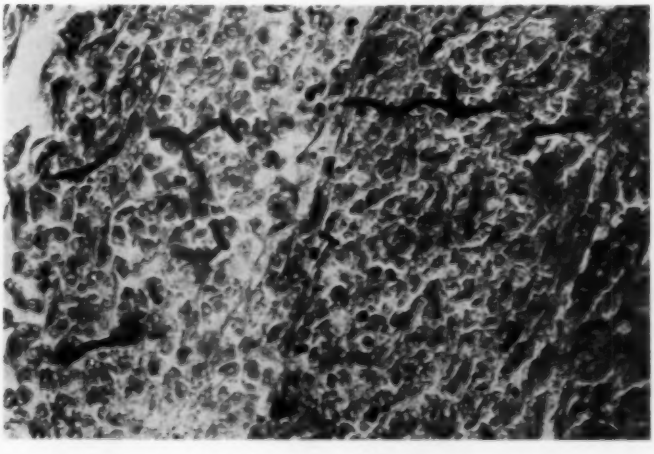

Fig. 7.—Hyphae of Hormodendrum species at edge of granulomatous and caseous nodule formed in the subcutaneous tissue of a guinea pig, three weeks after inoculation. Note the similarity of the organisms to those seen in the tissue of the patient. Periodic acid-Schiff stain; reduced to 2/3 of mag. × 625.

Classification of the Fungus

A culture of the fungus was sent to Dr. Arturo L. Carrion,* in San Juan, Puerto Rico, who expressed the opinion that the organism should be classified as a species of Hormodendrum. Although the genus Hormodendrum has been well established as a group, the identification of species within this group is often difficult because the Hormodendrum is usually discarded as an unimportant contaminant, and consequently the morphology of various species is little known.

The fungus isolated from the present case resembles certain examples of Hormodendrum that have been associated with chromoblastomycosis,[1] but differs significantly from the species ordinarily cultured from cases of this disease (Hormodendrum pedrosoi, Hormodendrum compactum, and Phialophora verrucosa). It most closely resembles in morphological and cultural characteristics the fungus isolated from two cases of brain abscess that were studied by Emmons and were classified by him as a new species, Cladosporium trichoides.† Emmons rejected the name Hormodendrum for the genus and was unable to find a species that had been adequately described corresponding to his organism.

We are not qualified to settle matters of taxonomic nomenclature that are still matters of debate; therefore, we shall follow the lead of Conant [4] in his discussion of this controversial problem and retain the generic name Hormodendrum because of its widespread usage in describing the type of sporulation found in this group of fungi. Our designation of the fungus would then be Hormodendrum species, and we would recognize its close similarity to and probable identity with the fungus named by Emmons Cladosporium trichoides.

Comment

We believe that in the case reported here there is no doubt concerning the etiologic significance of the Hormodendrum species isolated from the patient. In support of this statement are the facts that (1) the fungus was observed in histologic sections of the original surgical specimen; (2) no bacteria were cultured from this lesion by routine methods; (3) a pure culture of the fungus was obtained repeatedly from the patient before death and, again, from the brain after death; (4) a limited but definite degree of pathogenicity for experimental animals was demonstrated, and (5) the morphology of the fungus was similar in the tissue of the patient and in the lesions in animals.

Despite an intensive search, both clinically and at necropsy, for some lesion that might represent a portal of entry for this fungus, none could be found. The only conceivable means by which it could have gained access to this portion of the central nervous system would be by way of the blood stream. It may be pointed out in this connection that the patient had a record of numerous episodes of superficial trauma. It is possible that the fungi, introduced into the tissues at the site of such trauma, may have produced an insignificant lesion that served as a focus for hematogenous spread, and then this lesion may have healed before symptoms due to the cerebral lesion became manifest.

The similarity of this case to the one reported by Binford and associates [2] and the one reported by King and Collette,[3] both in 1952, is quite close, not only with respect to the fungus isolated but also in many features of the clinical course and type of lesion. In all three cases the brain abscess was an isolated lesion, with no portal of entry apparent. The patient described by Binford and associates was reported as being well two years after the craniotomy; the patient described by King and Collette died after a short period. The present case was intermediate between the two in this respect. The tissue reaction is also quite similar.

It appears to be well established by these

* Carrion, A. L.: Personal communication, April, 1951.

† References 2 and 3.

cases that in rare instances fungi of the genus Hormodendrum may be pathogenic for man and produce brain abscesses without producing significant superficial lesions resembling those of chromoblastomycosis. Furthermore, no reference can be found indicating that chromoblastomycosis shows any striking tendency to become disseminated, or to produce metastatic brain abscesses.

Summary

A third case of an isolated brain abscess due to a fungus Hormodendrum species (probably identical with Cladosporium trichoides, Emmons, 1952), is reported. Like the two other cases, this case was characterized by a granulomatous reaction with extensive necrosis in the brain and without any lesions elsewhere in the body related to the fungus. Like one of the two previous cases, it ended fatally, due to extension of the infection to adjacent brain tissue. The fungus isolated from this case had a mild degree of pathogenicity for laboratory animals. Fungi of this group must be considered as pathogenic for man in rare instances.

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Observations on the Adrenal of the Premature Anencephalic Fetus

JOHN NICHOLS, M.D., New Haven, Conn.

It is well documented that the adrenal cortices of the term anencephalic fetus are small. Indeed, not a few autopsy protocols record them as being absent. It is commonly presumed that this atrophy is related to the pituitary, which is deficient or absent in these cases; thus, it is implied that a deficiency of fetal pituitary adrenocorticotropic hormone (ACTH) is responsible for the atrophic adrenal cortex. The single report by Meyer [1] indicating the adrenals of a premature anencephalic fetus of five months' gestation to be normal despite absence of the pituitary is not well known. I had recently the good fortune to obtain the second premature anencephalic fetus of five months' gestation with absent (?) pituitary and normal adrenals, as well as a malformed fetus of five months' gestation with absent pituitary and normal adrenals. The purpose of this paper is to record these findings and to discuss the fetal-maternal-pituitary-adrenal relationships in light of current knowledge on these subjects.

Report of Cases

The first case is that of a premature anencephalic fetus without a placenta submitted from an outside hospital and on which no significant data are available except that the pregnancy was considered to be normal and in the fifth month of gestation. After the fetus had been dissected by the previous prosector with all of the thoracic and some of the abdominal viscera removed, the weight was 325 gm. and the crown-rump length was 14 cm. If the head had been normal, no doubt this length would have been increased. Figure 1 shows the fetus after I had partially reconstructed it. The adrenals were found in

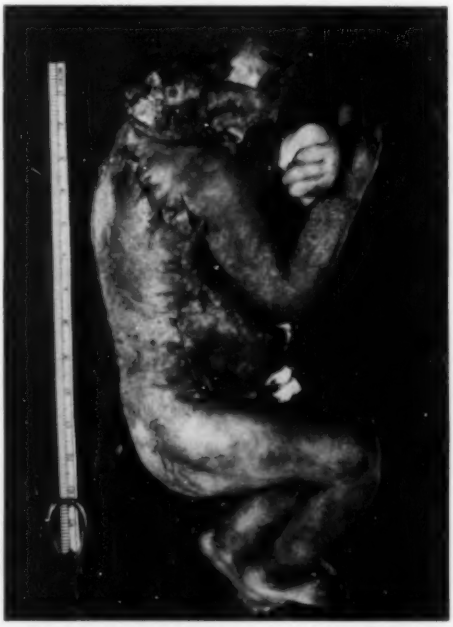

Fig. 1.—Anencephalic fetus of five months' gestation after reconstruction by me. Weight 325 gm.

their normal position and appeared to have approximately the normal mass ratio to the kidneys, as judged by the portions of the kidneys remaining in situ. The brain had been the site of considerable dissection by the previous prosector, and no pituitary tissue was found despite a careful inspection with a stereomicroscope and serial sections of the region. No sella turcica was found; only a flat plate was present posterior to the optic foramina. However, the failure to find pituitary tissue in this case cannot be considered

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conclusive evidence of its absence, because only serial sections of the intact region could have given this information.

Microscopic examination of sections of the adrenals stained with hematoxylin and eosin revealed the presence of a normal cortex for this stage of development. Figure 2 shows just beneath the capsule a layer several cells thick which is composed of relatively well-defined cells with a slightly basophilic cytoplasm. This layer is similar to the definitive cortex of the newborn. The remainder of the cortex is composed of a mass of large cells with clear cytoplasm which stain poorly; this represents the normal appearance of the transitional cortex of the newborn. The histological features of the fetal and the neonatal adrenal cortex are well known and are not the subject of this paper. Frozen sections stained with Sudan III revealed a small amount of lipid material in the definitive cortex and none in the transitional cortex. Schultz stains revealed this sudanophilic material to contain cholesterol and to have the same distribution as the sudanophilic material. The histological picture and distribution of lipids are identical with those for "normal" adrenals obtained for comparison from abortions in the fifth month of gestation. The adrenals of this premature anencephalic fetus had a combined weight of 0.63 gm. and a total cholesterol content of 0.49%. The adrenals of a "normal" fetus of five months' gestation had a combined weight of 0.70 gm. and a total cholesterol content of 0.54%. Figure 3 shows for comparison the adrenals from the normal fetus of five months' gestation (*A*) and the adrenals from the anencephalic fetus of five months' gestation (*C*). Included for comparison are the ad-

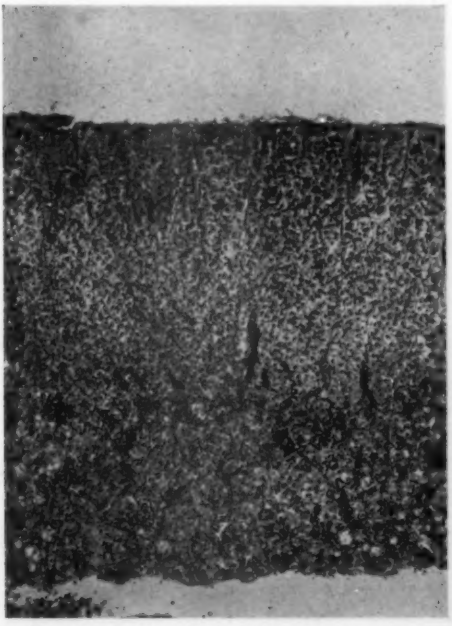

Fig. 2.—Section of adrenal cortex of premature anencephalic fetus shown in Figure 1. Note the relatively well-defined definitive cortex a few cell layers thick beneath the capsule and the poorly defined inner fetal zone. Hematoxylin and eosin stain.

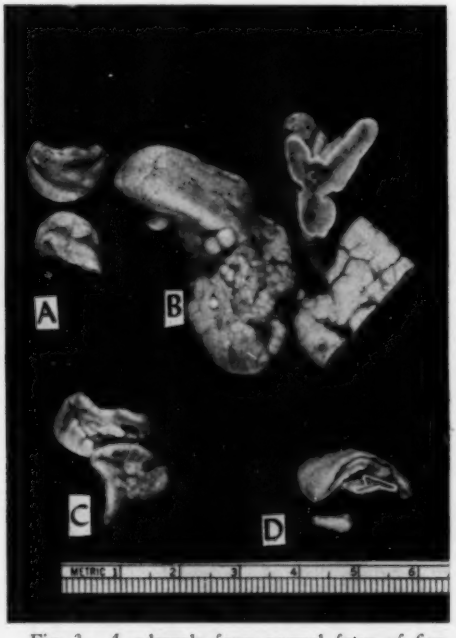

Fig. 3.—*A*, adrenals from normal fetus of five months' gestation; weight 0.70 gm.

B, adrenals from normal term fetus; weight 9.6 gm.

C, adrenals from premature anencephalic fetus of five months' gestation shown in Figure 1; Weight 0.63 gm.

D, adrenals from an anencephalic fetus at term; weight 0.45 gm.

renals from a term infant dying shortly after birth of intracranial hemorrhage (*B*), having a combined weight of 9.6 gm. and a total cholesterol content of 1.15%, and the adrenals from a term anencephalic fetus (*D*) weighing 0.45 gm. and having a total cholesterol content of 2.13%. The quantitative cholesterol determinations were done for me by Dr. John Forbes according to the method of Outhouse and Forbes.[2] Histological examination of these adrenals reveals those of the normal infant to have the sudanophilic material limited to the definitive subcapsular zone with the inner transitional zone free of lipids, while those of the term anencephalic fetus are devoid of transitional cortex and the lipids are present in the entire definitive cortex, which stains with the Sudan and the Schultz reagent with the same intensity as the definitive cortex of the normal term infant. It is noted that the adrenal from the anencephalic fetus at term has a higher percentage value of cholesterol than does the normal-term adrenal. This may be relative, and not absolute, because the normal adrenal at term has a considerable mass of transitional cortex free of cholesterol, but this mass contributes to the total weight, thereby reducing the percentage value for cholesterol. In other words, the definitive cortex of the normal newborn and the adrenal tissue of the anencephalic at term probably have the same cholesterol content.

It can be seen that the adrenal of the anencephalic fetus at term (in this case) is smaller than the adrenal of the anencephalic fetus at the fifth month of gestation and that the adrenal of the anencephalic fetus at term has the higher cholesterol content. The normal infant at term, weighing 2460 gm. and having adrenals weighing 9.6 gm. with a cholesterol content of 1.15%, has, therefore, 0.11 gm. of adrenal cholesterol. The anencephalic fetus at term, weighing 2400 gm. and having adrenals weighing 0.45 gm. with a cholesterol content of 2.13%, has, therefore, 0.009 gm. of adrenal cholesterol. It can be seen that the infant at term has approximately 20 times the adrenal mass but only about 12 times as much cholesterol as the anencephalic fetus at term. These figures are representative of a larger, unpublished series of mine.

The second case is that of an abortion at the fifth month of gestation, the previous history of which was entirely normal and which is shown in Figure 4. The membrane bones of the skull were

Fig. 4.—Premature fetus of five months' gestation. Note fusion of margin of placenta with skin of head. Cranial cavity filled with hemorrhagic viscous fluid. Pituitary absent.

not present, and the skin of the head was fused with the membranes of the placenta. The resulting sac, opened after fixation with formalin, was found to contain a thick hemorrhagic, mucinous fluid, which drained away, leaving bare bone without any nerve structures except the spinal cord and stumps of the various cranial nerves in their foramina. This would imply that the brain had formed and later degenerated. Serial sections of the sella region failed to reveal any pituitary tissue in the cranial cavity, in the persistent Rathke's canal, or in the roof of the pharynx. The adrenals were grossly normal and had a combined weight of 0.60 gm., with a total cholesterol content of 0.45%. Histological examination revealed them to be identical with the adrenals

from the normal and anencephalic fetuses of five months' gestation.

Comment

From the foregoing, it seems conclusive that Meyer's original report of a premature anencephalic fetus at five months' gestation with normal adrenal cortices is correct. The adrenal from the anencephalic fetus at term seems to have the normal definitive cortex, as found beneath the capsule of the adrenals in the normal term infant, the atrophy being due to the involution of the fetal zone, which has taken place after the fifth month of gestation. The files of the pathology department of the Medical College of Virginia contain an anencephalic fetus of seven months' gestation with atrophic adrenals. Presumably, therefore, the atrophy occurs between the fifth and the seventh month of gestation. The fetal zone has been the subject of much speculation [3] and has recently been reviewed by Lanman.[4] Suffice it to say that none of the various proposed functions are agreed on by the many writers. Indeed, the zone may not have a function; at least it does not contain any stainable cholesterol, as does the definitive cortex. It is recalled that these two zones have different histories; thus, the fetal cortex migrates from the intermediate cell mass of the lateral-plate mesoderm at the 10 mm. stage, while the definitive cortex migrates from the same site at the 12 mm. stage, simultaneously with the invasion of the fetal cortex by neural crest cells destined to form the medulla.[5] However, the absence of cholesterol in the fetal cortex (presuming the cortical hormone to be formed from cholesterol) cannot be taken as conclusive evidence of lack of function because the adrenal cortex of such animals as the hamster normally contains no stainable cholesterol. A suggestion that the fetal cortex does secrete hormone(s) could be deduced from the reports of Gardner and Walton * that the plasma of the premature infant has a higher level of cortical hormones than the infant at term and that the infant at term has a higher titer than does the normal maternal circulation. However, these high titers of plasma corticoids may not arise from the fetal cortex, as suggested by Gardner and Walton, for DiGeorge † reports that the term anencephalic fetus with atrophic adrenals has a high level of plasma 17-ketosteroids.

The widely held supposition that the atrophic adrenal of the term anencephalic is due to the deficient pituitary associated with these cases is probably not tenable, for at least two reasons. First, Angevine,[8] in a carefully controlled study with serial sections of the pituitary region of the anencephalic at term, found varying amounts of pituitary tissue always present, and these varying amounts of pituitary tissue were not related to the amount of adrenal cortex present. Second, prolonged massive treatment with corticotropin of a newborn infant for retrolental fibroplasia did not delay the normal involution of the fetal zone,[4] as observed at autopsy.

The human fetal zone is often compared to the X-zone of the mouse adrenal, despite many divergent features. The main points of similarity are the same general morphological appearance and anatomical location and the fact that both are transient structures in the early life of the organism.

Thus, the X-zone of the mouse adrenal appears at 10-14 days of age and in the male begins involution at 28 days, being complete at 40 days, whereas in the female it persists until 200 days, or establishment of pregnancy if the latter comes first. It is noted that between 28 and 40 days the male mouse passes through puberty, with the elaboration of androgens, and when the female mouse becomes pregnant, the ovary elaborates androgens under stimulation of gonadotropins. Thus, gonadectomy in the male mouse will cause the X-zone to persist, while administration of androgen to the prepubertal male, castrate male, and young

* References 6 and 7.

† DiGeorge, A. M.: Personal communication.

female will cause immediate involution. It seems clear, therefore, that the X-zone of the mouse is related to androgens.[9] Further, it is related to gonadotropins because administration of pituitary gonadotropin (LH) to a male mouse castrated after puberty and with an involuted X-zone will cause regeneration of the X-zone.[9] The fact that gonadotropins cause proliferation of the X-zone in the mouse and that chorionic gonadotropins (LH in character) are highest in early pregnancy in man, when the adrenal is actively growing, and because adrenal involution takes place later when chorionic gonadotropins are withdrawn (birth) has caused Chester Jones[9] to set forth the idea that the atrophic adrenal of the anencephalic is due to a possible defect in the placenta, and not to a defect in the pituitary. Unfortunately, the placenta is rarely examined in anencephaly. The placenta in the second case of this paper was normal on histological examination apart from the fusion of the membranes at the margin with the skin of the head. Judging from the few reports in the literature, anencephalics do not have a tendency to prematurity or abortion; this would seem to make any possible defect in the pituitary a minor one. Indeed, Malpas[10] gives anencephaly as one of the few absolute causes for postmaturity.

One must consider the evidence for the transfer of ACTH across the placenta; in fact, ACTH has been isolated from the chorionic villi,[11] indicating another function for this structure. It has been reported by Sheehan and Murdoch[12] that hypopituitarism patients (Sheehan's syndrome) who succeed in becoming pregnant often have ameloriation of the symptoms as long as the pregnancy lasts, thereby possibly indicating placental formation or transfer of ACTH. Smith[13] presents another line of evidence indicating that the placenta of the monkey is a second source of ACTH. It is reported that adrenalectomy of a pregnant rat results in hypertrophy of the fetal adrenals,[14] presumably due to the increased elaboration of ACTH by the maternal pituitary and placental transfer thereof, because prior hypophysectomy of the mother prevents this hypertrophy of fetal adrenals. Exogenously administered corticotropin can cross the placental barrier and cause hypertrophy of the fetal adrenals, as reported by Jones and associates.[15] Recent work by Chester Jones and Christianson,‡ however, indicates that in the rat the hypertrophy of fetal adrenals after maternal adrenalectomy is due to fetal pituitary action and not, as might be expected, to the action of maternal ACTH crossing the placenta. This is in keeping with Jost's[16] observations on fetal gonadotropins.

It would appear that in the rat ACTH of fetal origin can cross the placenta and help maintain the maternal adrenals, because hypophysectomy of a pregnant rat causes less atrophy of the maternal adrenal glands than does hypophysectomy of a nonpregnant rat. It has already been established that the fetal pituitary does contain ACTH.§ Chester Jones and Christianson consider, however, that equivalent adrenal atrophy is found in hypophysectomized pregnant and nonpregnant rats. This evidence that the fetal pituitary of the rat and human is functioning is somewhat contrary to the findings of Jailer,[19] who reports that the pituitary and adrenal of the newborn rat react sluggishly to epinephrine. It seems definitely established that the adrenal of the newborn does function when considered in light of the findings of Gardner and Walton that the premature and newborn infant have higher levels of corticoids than does the mother. Chester Jones and Christianson consider that the fetal rat pituitary does not necessarily function normally, but can be made to do so by such procedures as maternal adrenalectomy.

‡ Jones, Chester I., and Christianson, M.: Foetal-Maternal Relationships in the Rat, unpublished study.

§ References 17 and 18.

Summary

The second case of a premature anencephalic fetus of five months' gestation with normal adrenals and absent (?) pituitary is recorded, together with a case of a malformed fetus of five months' gestation with absent pituitary and normal adrenals. It is concluded that atrophic adrenal of the anencephalic fetus at term has more cholesterol than does the adrenal from the anencephalic fetus of five months' gestation. The normal fetus at term has 20 times the mass of adrenal tissue and 12 times as much adrenal cholesterol as does the anencephalic fetus at term. From a survey of the literature it is probable that the fetal zone does form cortical hormones and from the findings in this paper this activity is independent of adrenocorticotropic hormone.

NOTE:—After this paper was prepared for publication, an excellent review appeared by Benirschke and associates.[20]

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Fatty Change of the Myocardium in Early Experimental Infarction

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Correlation of the results obtained by different histochemical procedures allows one to obtain a composite and sequential picture of some of the many metabolic disturbances taking place during the early stages of experimental myocardial infarction. As a part of such a correlative study we have previously described the use of the periodic-acid-Schiff (PAS) technique to demonstrate the marked reduction of myocardial glycogen that occurs about one hour after ligation of a coronary artery.[19] The PAS method also reveals that soon after the glycogen is diminished the affected myocardial fibers undergo a peculiar degenerative change, characterized by an intense and progressive increase in the staining reaction. The nature of the degenerative material that stains with PAS is unknown, but it is not glycogen, since digestion of the tissue with α-amylase before staining does not affect the reaction. Other histochemical procedures, which were carried out on other sections of the same samples of tissue, have revealed additional chemical alterations in the ischemic myocardium, and the purpose of this paper is to describe one of these changes, namely, the accumulation of neutral fat a few hours after the onset of ischemia.

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From the Department of Pathology, Northwestern University Medical School.

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Methods

A total of 40 mongrel dogs were used for the experiments. In 29 animals the anterior descending branch of the left coronary artery was ligated just proximal to the apical branch.* This procedure produced small infarcts in a predictable location which were suitable for histochemical studies of the changes occurring during the very early stages of ischemia, when the usual morphological evidences of infarction had not yet developed. Samples of ischemic myocardium were collected 20 and 25 minutes after ligation and at hourly intervals during the first six hours. Samples were also taken at 12 hours and at 1, 2, 4, 7, 14, 28, and 42 days. In five additional animals the ligature was placed high on the left circumflex coronary artery,[5] and samples of the myocardium were collected at 4, 6, and 12 hours after the occlusion was accomplished.

Three types of control tissues were studied: (1) nonischemic myocardium from six normal dogs with and without sham operation; (2) samples of nonischemic myocardium from the left ventricle of the experimental animals, and (3) samples of normal myocardium allowed to autolyze in moist chambers at 27 C.

Both experimental and control dogs were killed by air embolism or exsanguination under pentobarbital anesthesia, and samples of myocardium were collected immediately and placed in large quantities of neutral formalin solution buffered with phosphate. Frozen sections were made of many different samples of tissue from each dog and stained with oil red O dissolved in propylene glycol and counterstained with hematoxylin.[8] Other samples of tissue were embedded in paraffin and stained with hematoxylin and eosin, Heidenhain's trichrome method, and other methods.

* References 18, 19.

Results

Normally a small number of fine globules situated at the nuclear pole of the myocardial fibers stain with oil red O (Fig. 1).

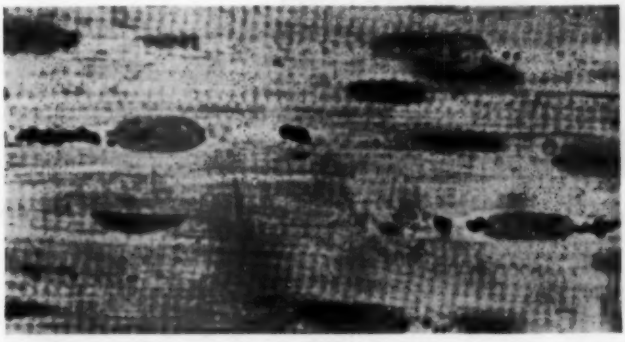

Fig. 1.—Frozen section of formalin-fixed normal dog myocardium, stained with oil red O and counterstained with hematoxylin. Lipochrome pigment granules in the sarcoplasm at the poles of nuclei are stained dark with oil red O. Very few small droplets of fat (upper right and lower left corners) appear in the normal myocardium. This animal was subjected to sham operation. Green filter; reduced to 88% of mag. ×774.

These globules lie in the sarcoplasm and are probably part of the complex of lipochrome pigment. In most dogs this is the only material that stains with oil red O, but occasionally a few very minute droplets of fat may be seen elsewhere in the sarcoplasm. For this reason, before coming to conclusions about the meaning of the fat deposits, it is necessary to compare the slides from the experimentally induced lesions with appropriate controls from uninvolved areas of myocardium. In addition to fat droplets in the fibers, the plasma within blood vessels usually stains uniformly pale orange-red.

Distribution of Neutral Fat in Experimental Myocardial Infarction

Time	No. of Animals	No. with Fat in Ischemic Myocardial Fibers
Hours		
½	2	0
1	4	3
2	2	2
3	2	2
4	5	5
5	3	3
6	6	6
12	2	2
Days		
1	1	1
2	2	2*
4	1	1*
7	1	1*
14	1	1*
28+	2	0*
Total	34	29

* Fat present in some myocardial fibers immediately adjacent to the infarct.

The accompanying Table shows the results of the experiments. For as long as one hour after ligation of a coronary artery no abnormal accumulation of fat could be demonstrated in the ischemic myocardial fibers, but after this time fat appeared in some of the affected fibers. At the end of six hours of ischemia all animals showed a marked fatty change, which persisted as long as two weeks. The earliest change was either a faint homogeneous pink staining of the myocardial fibers or the appearance of very fine fat droplets. For as long as the fat persisted, it occurred in the form of discrete small or large droplets and was located in the sarcoplasm (Figs. 2 and 3). Animals that survived 28 and 42 days showed no fatty change except in occasional fibers surrounding the scar tissue.

In the early stages a small quantity of fat was demonstrable in only a few fibers, but in the later stages the number of fibers affected and the amount of fat deposited both increased markedly. However, even when maximal fatty change had occurred, it was never uniform but always patchy (Fig. 4). Close examination of the fibers that

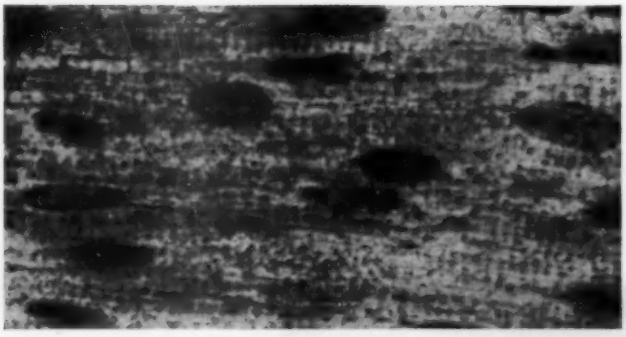

Fig. 2.—Frozen section of formalin-fixed myocardium, stained with oil red O, showing an increase in fat droplets in a six-hour infarct. Green filter; reduced to 88% of mag. × 774.

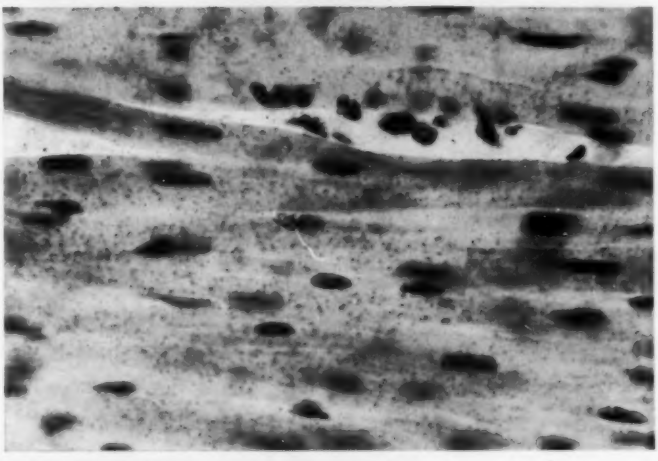

Fig. 3.—Oil red O staining of fat droplets in a six-hour infarct. Nuclei counterstained with hematoxylin. Photograph was taken from the same section as for Figure 2 but from another area. Green filter; reduced to 88% of mag. × 580.

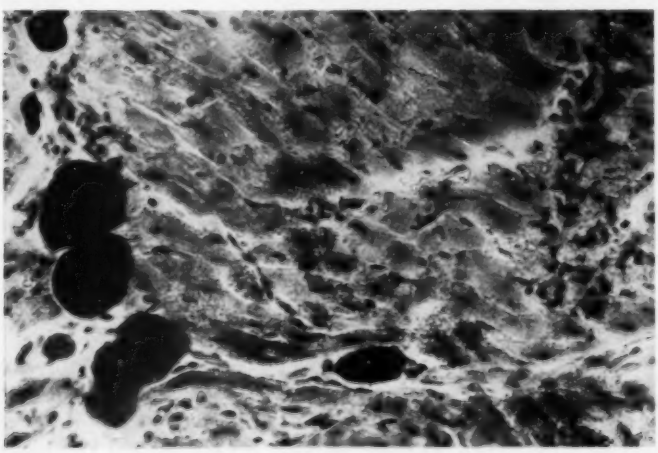

Fig. 4.—Oil red O staining of a four-day-old infarct. The fat droplets in the partially injured myocardial fibers show a patchy distribution. The large masses of darkly stained material at the left are fat cells. Green filter; reduced to 88% of mag. × 500.

contained little or no fat revealed evidence of severe cellular injury, such as swelling, architectural disorganization, and nuclear pyknosis. This observation suggests that fat accumulated in significant amounts only in cells that, although injured, were still living and not in cells that died rapidly.

After two days macrophages containing

cytoplasmic fat droplets were noted in the affected area. Animals that survived 28 and 42 days showed no fatty change except in a few fibers in the surrounding scar tissue.

Effects of Autolysis.—In another series of experiments the effect of postmortem autolysis on the development of fatty change was tested. Hearts from anesthetized normal (control) dogs were placed in moist chambers and maintained at 27 C for as long as five hours. Samples of the left ventricle were removed at one, three, and five hours and stained for fat. The fat of the lipochrome pigment persisted unaltered for the entire duration of the experiment, but no other stainable fat appeared. Infarcts of one hour's duration were also allowed to autolyze under the same conditions. These tissues did not develop any further fatty change than was already present.

Comment

Only a few other investigators have made detailed studies of the fatty changes that occur in experimental myocardial infarction. Karsner and Dwyer, using Sudan III to demonstrate the neutral fat in dog infarcts produced by high ligation of the anterior descending branch of the left coronary artery, did not observe fatty changes in the myocardial cells until 24 hours after the ligation,[6] when the muscle cells showed hyaline necrosis. Our ability to demonstrate the fat at an earlier period was probably due to extensive sampling of infarcts of known location and the use of oil red O in propylene glycol, a dye which is more sensitive than Sudan III for the demonstration of slight accumulations of lipid. Kent and Diseker, using oil red O and Sudan IV, observed small droplets of fat nine hours after they placed a ligature on the left coronary artery of dogs.[7] Karsner and Dwyer noted that after 48 hours fat was present in sharply demarcated areas corresponding to the zones of necrosis. At the end of 11 days the fat had disappeared from the necrotic areas, but was still present at the margin of the infarct in the form of globules, some of which were in extracellular positions and some in the cytoplasm of fibroblasts. After 18 days no fat was present.

Lipid changes have also been observed in human myocardial infarcts.† In a study of healing infarcts, Mallory, White, and Salcedo-Salgar [8] observed fatty change in the necrotic fibers, especially at the edges of the infarct. They attributed the occurrence of the fat to the state of the myocardium previous to infarction, although they did not give evidence to support their belief. Thus they assumed that if the infarcted myocardium previously had an insufficient circulation, fatty degeneration would occur and the infarcted muscle would contain fat droplets, whereas if the muscle were entirely normal before infarction it would show only a small amount of fat.

The literature also contains several reports describing the development of fatty change in the myocardium during hypoxia, without actual infarction. Büchner, for example, found small areas of myocardial necrosis and fatty degeneration in the hearts of patients who had angina pectoris during life but no infarct at autopsy.[1] Müller and Rotter found focal fatty change in the myocardium of German aviators dying suddenly during high altitude flights.[9] Fatty change has also been observed in cats, rabbits, rats, and guinea pigs under various experimentally produced conditions of hypoxia.‡

The patchy nature of the fat changes in our animals is of interest, for it indicates that not all the myocardial fibers in the infarct were injured to the same degree. The evidence to support this belief is not yet complete, but histologic observations of the ischemic myocardium suggest that cells that died rapidly did not accumulate fat, whereas cells that died slowly, or escaped irreversible injury, did accumulate fat. Presumably, the partially injured fibers were

† References 8, 12.

‡ References 4, 11, 13, 16, 17.

Fig. 5.—Formalin-fixed, paraffin section of a two-day-old infarct stained with the periodic-acid-Schiff method after treatment with amylase; not counterstained. The dark areas indicate injured fibers and the light areas indicate normal fibers. Green filter; reduced to 88% of mag. × 21.

sufficiently intact metabolically to accept lipids from the plasma but were unable to metabolize them at a rate great enough to prevent the accumulation of neutral fat in the cell. Later, some of the partially injured fibers no doubt died, but that some of them survived can be shown in histological preparations of a well-developed infarct several days or more old (Fig. 5). Thus, most small experimental infarcts in the dog seem to be heterogeneous, rather than homogeneous, and are composed of a mixture of dead, injured, and living myocardial fibers.

The source of the fat that was deposited in the ischemic myocardial fibers in our experiments is not certainly known. The finding that stainable neutral fat does not appear in the autolyzing dog myocardium suggests that the fat is brought to the heart by the circulating blood. This view is also supported by Dible's chemical data on phosphorus poisoning in guinea pigs, which shows that the fat in the injured myocardium of these animals was exogenous in origin.[2] However, the possibility cannot be excluded that some of the fat seen in ischemic myocardial fibers results from unmasking of cellular lipids.

Summary

Oil red O dissolved in propylene glycol was used to study the distribution of neutral fat in normal dog heart and in experimental myocardial infarcts of predictable location. A slight and variable increase of neutral fat in ischemic myocardium was first noted one to three hours after occlusion of a coronary artery. After four to six hours the changes became more marked and increased steadily during the next 18 hours. The fat persisted in apparently viable myocardial fibers within and around the infarct for the next 14 days and then began to diminish.

In autolysis experiments neutral fat did not increase in the myocardial fibers.

These studies suggest that, under the conditions of the experiments, neutral fat appeared in appreciable quantity within the sarcoplasm of fibers partially injured by the ischemia. Most of these fibers were probably reversibly injured, although some of them eventually died after accumulating large amounts of fat. Fibers injured irreversibly at the time of occlusion did not accumulate much neutral fat. The fat probably came from the circulating plasma lipids.

Zigmund Zudyk, Dorothy Foss, and Naomi Lemkey gave technical assistance.

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The Generalized Shwartzman Phenomenon in Rats

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The morphologic changes produced in rabbits by the intravenous administration of Gram-negative bacterial endotoxin have been described in previous papers.* With two appropriately spaced intravenous injections of endotoxin, bilateral renal cortical necrosis is regularly produced, and this lesion has been termed the "characteristic and identifying" lesion of the generalized Shwartzman phenomenon.[1] Chronologic study of the development of this renal lesion has shown that it is produced by occlusion of the glomerular capillaries by a hyaline material with the morphologic and tinctorial properties of fibrinoid.† Other studies have shown that a similar renal lesion can be produced by the intravenous administration of certain high molecular weight acidic polymers (sodium polyanethosulfonate [Liquoid], dextran sulfate, and polyvinyl alcohol sulfonate) when these substances are given in conjunction with endotoxin or, in the case of sodium polyanetholsulfonate, when given alone in sufficiently large amounts.‡

The biologic activity of these acidic polymers with respect to fibrinogen,§ the occurrence of an altered form of fibrinogen in rabbits given Gram-negative endotoxin,‖ the abrupt depletion of this altered fibrinogen following the injection of one of these polymers,‖ and the synergistic action of these polymers when given with endotoxin,‡ strongly suggest that fibrinogen is involved in the formation of fibrinoid. The prevention of the renal lesion by the administration of large amounts of heparin ¶ further supports this theory.

These reports, however, have dealt exclusively with the changes in rabbits. Homma, in 1952,[12] reported the production of a hemorrhagic skin reaction in mice by giving single or multiple intradermal injections of bacterial toxins, followed by a single intravenous injection of toxin 18 to 20 hours later. Endotoxins derived from Proteus vulgaris, Pseudomonas aeruginosa, and Escherichia coli were used.

Numerous reports refer to the resistance of rats to endotoxin. Sobel,[13] using several types of endotoxin, was unable to produce the local Shwartzman phenonenon in rats. Cameron and associates,[14] however, reported "severe" liver necrosis associated with hemorrhage and "conglutination and hyaline thrombi" in the hepatic sinusoids of rats given one intraperitoneal injection of a toxic fraction derived from Salmonella typhimurium (Bacterium typhi murium). The kidneys of these rats showed only congestion of the large vessels and occasional small extravasations of red blood cells into the capsular spaces of the glomeruli. These workers also noted numerous "conglutina-

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* References 1-3.

† References 3, 4.

‡ References 5, 6.

§ References 7, 8.

‖ References 9, 10.

¶ Reference 6, 11.

tion thrombi" in the "smaller blood vessels and capillaries" of the lungs.

Piel and associates,[15] in describing certain renal lesions produced in rats by the intravenous administration of heterologous hyperimmune antiserum, noted that an occasional lesion resembled the renal changes of the generalized Shwartzman phenomenon, but it was emphasized that this reaction had never been regularly produced in the rat. Because of the apparent limitation of this reaction to the rabbit, an animal noted for exaggerated responses in many ways, the possible relationship of the Shwartzman phenonenon to human disease has been questioned by some.

These considerations, therefore, led us to investigate the effects produced in rats by the intraperitoneal administration of Gram-negative bacterial endotoxins in conjunction with the acidic polymer sodium polyanetholsulfonate (Liquoid), and this paper deals with the production and prevention of the generalized Shwartzman phenomenon in the rat.

Materials and Methods

Two hundred ninety-eight albino rats of the Sprague-Dawley strain of both sexes and varying weights were used in the experiments. They were fed Purina Fox Checkers and had free access to water.

Meningococcal and E. coli endotoxins were used in the experiments. The meningococcal toxin was prepared in the manner previously described.[1] E. coli toxin, prepared by a modification of the Boivin technique,[16] was obtained from Dr. R. T. Smith. Sodium polyanetholsulfonate (Liquoid) was obtained from Hoffmann-La Roche, Inc. These materials were dissolved in sterile, pyrogen-free, isotonic saline in such concentration that 1 cc. was injected at each dosage level. Heparin sodium was obtained from The Upjohn Company.

The heparin-precipitable fraction[9] was determined by adding 0.1 cc. (1 mg.) of heparin sodium to 5 cc. of blood. This was centrifuged at 1800 rpm for 20 minutes, and the plasma was drawn off and refrigerated at 4 C for two hours.

Because of the simplicity and the saving in time, all injections were given intraperitoneally.

The animals that did not die were killed 24 to 48 hours after the last injection. Postmortem examination was performed, and the tissues were fixed in 10% neutral formalin. Blocks were routinely taken from the heart, lungs, liver, kidneys, spleen, adrenals, and pancreas, and, in about one-third of animals, from the brain, testes, muscle, and intestine. These blocks were embedded in paraffin in the usual manner and sectioned at 7μ. The hearts were cut so as to show the interventricular septum and the valves. The sections were routinely stained with hematoxylin and eosin, and additional selected sections were stained with Mallory's phosphotungstic acid hematoxylin, toluidine blue, and the periodic acid-Schiff method.

Results

Morphologic Changes

The morphologic changes observed after the intraperitoneal administration of endotoxin and sodium polyanetholsulfonate, alone or in varying combinations, are summarized in Tables 1-5. From these Tables it may be seen that there was a definite increase in the number of animals which died within 24 hours after a single simul-

TABLE 1.—*Incidence of Lesions Following Intraperitoneal Injection of Endotoxin or Sodium Polyanetholsulfonate (Liquoid) Alone*

Procedure	Amount	No. Animals	No. Dead (24 Hr.)	Fibrinoid Lesions			Liver	Adrenal
				Kidney	Heart	Spleen	Necrosis	Hemorrhage
Meningococcal toxin	0.10 cc.	10	1	1	1	1	2	0
E. coli toxin	0.10 cc.	10	0	0	1	2	2	0
Liquoid	5 mg.	10	0	0	0	0	1	0
Liquoid	10 mg.	15	1	7	3	1	2	2
Liquoid	20 mg.	10	2	8	0	1	0	1

TABLE 2.—*Incidence of Lesions Following Intraperitoneal Injection of Meningococcal Endotoxin and Sodium Polyanetholsulfonate (Liquoid) (Simultaneous)*

Toxin, Cc.	Liquoid, Mg.	No. Animals	No. Dead (24 Hr.)	Fibrinoid Lesions			Liver Necrosis	Adrenal Hemorrhage
				Kidney	Heart	Spleen		
0.025	5	10	3	3	2	0	4	3
0.05	5	10	3	4	2	2	4	2
0.10	5	10	6	1	1	0	11	2
0.05	10	10	6	7	0	0	7	4
0.10	10	10	6	8	0	1	7	6

TABLE 3.—*Incidence of Lesions Following Intraperitoneal Injection of E. Coli Endotoxin and Sodium Polyanetholsulfonate (Liquoid) (Simultaneous)*

Toxin, Cc.	Liquoid, Mg.	No. Animals	No. Dead (24 Hr.)	Fibrinoid Lesions			Liver Necrosis	Adrenal Hemorrhage
				Kidney	Heart	Spleen		
0.025	5	10	2	2	0	0	0	0
0.05	5	10	2	2	1	1	0	0
0.10	5	10	5	6	0	0	3	0
0.05	10	10	6	6	0	1	3	6
0.10	10	10	4	7	0	0	4	4

TABLE 4.—*Incidence of Lesions After Intraperitoneal Injection of Endotoxin Followed in Two Hours by Sodium Polyanetholsulfonate (Liquoid)*

Toxin, Cc.	Liquoid, Mg.	No. Animals	No. Dead (24 Hr.)	Fibrinoid Lesions			Liver Necrosis	Adrenal Hemorrhage
				Kidney	Heart	Spleen		
Meningococcal 0.05	5	10	3	4	3	6	5	4
Meningococcal 0.10	5	10	4	4	2	3	6	3
E. coli 0.05	5	10	4	3	0	3	2	0
E. coli 0.10	5	10	2	2	1	1	3	1

taneous injection of toxin and sodium polyanetholsulfonate, as compared with those which were given a single injection of either one of these substances alone. The pathologic changes in each group of animals were similar, but the incidence of the different lesions varied somewhat from group to group. Lesions involving the kidneys, liver, and adrenal glands were observed more frequently than were lesions of the other organs. The characteristic lesions were seen in the various organs as follows:

TABLE 5.—*Incidence of Lesions After Intraperitoneal Injection of E. Coli Toxin Followed in Eighteen Hours by Either Sodium Polyanetholsulfonate (Liquoid) Alone or Liquoid Plus E. Coli Toxin*

Toxin, Cc.	Second Injection		No. Animals	No Dead (24 hr.)	Fibrinoid Lesions			Liver	Adrenal
	Liquoid, Mg.	Toxin, Cc.			Kidney	Heart	Spleen	Necrosis	Hemorrhage
0.10	10	..	10	1	2	0	1	2	0
0.10	10	0.05	10	1	10	2	2	4	0

Kidney.—Gross bilateral cortical necrosis, similar to that described previously as occurring in rabbits, was observed in approximately 20% of all the animals used. The incidence of this lesion varied considerably, however, in different groups. It was not observed in any animal given endotoxin alone, but occurred in 60% of those given 20 mg. of sodium polyanetholsulfonate alone and in 40%-50% of those given toxin and sodium polyanetholsulfonate together in the largest dosage levels used.

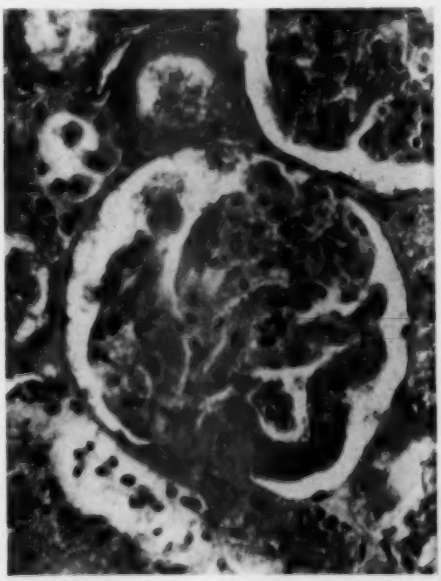

Fig. 1.—Glomerulus from the kidney of a rat killed 24 hours after the intraperitoneal administration of endotoxin and sodium polyanetholsulfonate (Liquoid). The glomerulus appears enlarged, and most of the capillaries contain fibrinoid material. Fibrinoid is also present within the lumen of the afferent arteriole. Hematoxylin and eosin stain; reduced to 91% of mag. × 400.

Microscopically, the renal glomerular capillaries showed the presence of hyaline acidophilic material within their lumens, with associated tubular necrosis and hemorrhages (Figs. 1 and 2). In animals which died six to eight hours following the administration of toxin and sodium polyanetholsulfonate the changes were less pronounced. Usually, only scattered glomeruli were involved, and no tubular necrosis was observed (Fig. 3). In addition to its accumulation in the glomerular capillaries, the material was occasionally observed in the media of larger renal arteries (Fig. 4). These changes were seen especially in the group of animals given an injection of both toxin and sodium polyanetholsulfonate 18 hours after an initial injection of toxin (Table 5). In all locations this hyaline material was intensely Schiff-positive (Figs. 4, 5), stained blue with toluidine blue, and purple to purple-orange with Mallory's phosphotungstic acid hematoxylin method. The morphologic and tinctorial properties of this material were identical with those of the hyaline fibrinoid material present in the kidneys of rabbits given Gram-negative endotoxin # or endotoxin in association with sodium polyanetholsulfonate.*

Heart.—Gross changes in the heart were infrequently observed and consisted of small areas of hemorrhage. Microscopically, approximately 15% of the hearts showed valvular hemorrhage and areas of myocardial hemorrhage and necrosis with surrounding mononuclear-cell reaction. In

References 1, 3.
* References 5, 6.

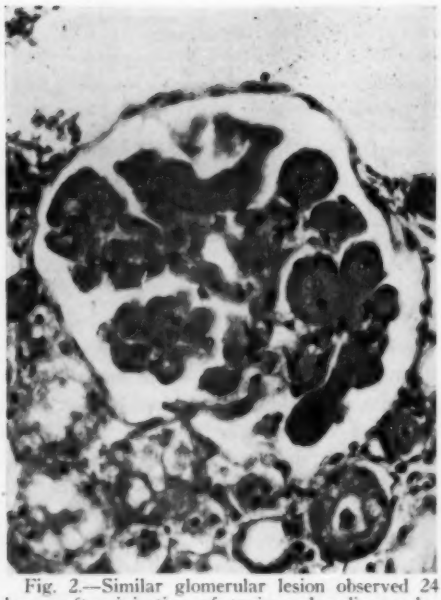

Fig. 2.—Similar glomerular lesion observed 24 hours after injection of toxin and sodium polyanetholsulfonate (Liquoid). There appears to be complete occlusion of nearly all capillary loops by extensive accumulations of fibrinoid. Hematoxylin and eosin stain; reduced to 91% of mag. × 400.

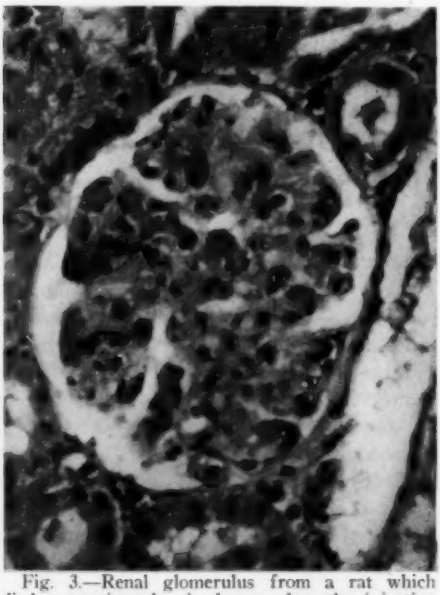

Fig. 3.—Renal glomerulus from a rat which died approximately six hours after the injection of toxin and sodium polyanetholsulfonate. Scattered capillary loops are occluded by fibrinoid, but a majority appear free of this material. Hematoxylin and eosin stain; reduced to 91% of mag. × 400.

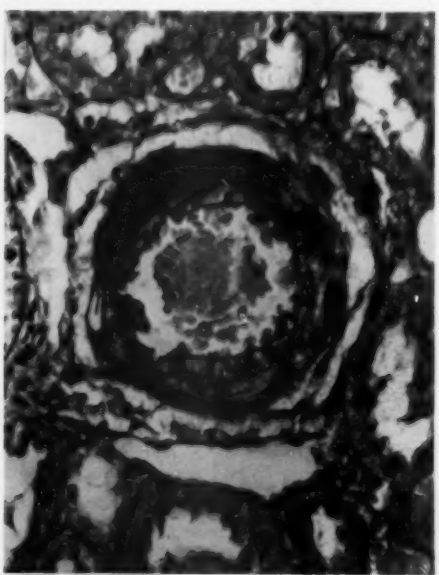

Fig. 4.—Section of a renal artery stained by the periodic acid-Schiff method, showing extensive deposits of Schiff-positive fibrinoid material within the media of the vessel; reduced to 91% of mag. × 400.

occasional animals hyaline thrombi were present in the intramural coronary arteries (Fig. 6). Fibrinoid was observed infrequently in the pericardium, coronary arteries, and heart valves of the animals. Along the pericardial surface and within the valves it was often accompanied by a cellular reaction of large mononuclear and Anitschkow-like cells. In the arteries it was usually observed to be just beneath the endothelium, appearing as a thin band of homogeneous eosinophilic material. Occasionally it appeared to protrude into the lumen of the vessel.

Spleen.—Gross changes in the spleen were infrequent and consisted only of enlargement and apparent congestion. No areas of necrosis were seen. Microscopically, in those animals that died four to eight hours after the injection of toxin and sodium polyanetholsulfonate, the sinusoids appeared to be intensely congested, but no other changes were noted. In animals that died at a later period, clumps of eosinophilic hyaline ma-

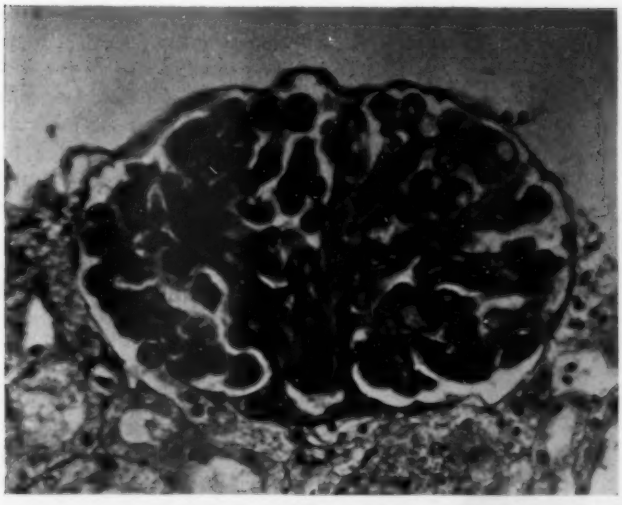

Fig. 5.—Renal glomerulus stained by the periodic acid-Schiff method, showing extensive deposits of Schiff-positive fibrinoid material in the capillaries. In some of the capillaries this material is closely applied to the basement membrane, producing a "wire-loop" appearance. × 400.

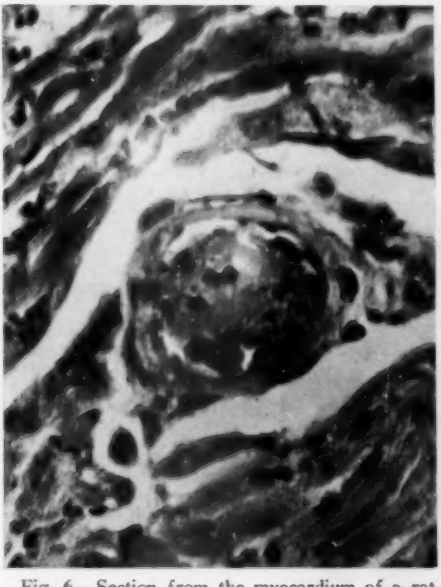

Fig. 6.—Section from the myocardium of a rat which died approximately 12 hours after injection of toxin and sodium polyanetholsulfonate, showing an intramural coronary artery that is occluded by a fibrinoid thrombus. At one point on the vessel wall the fibrinoid material appears to be continuous with the media of the vessel. Hematoxylin and eosin stain; reduced to 91% of mag. × 500.

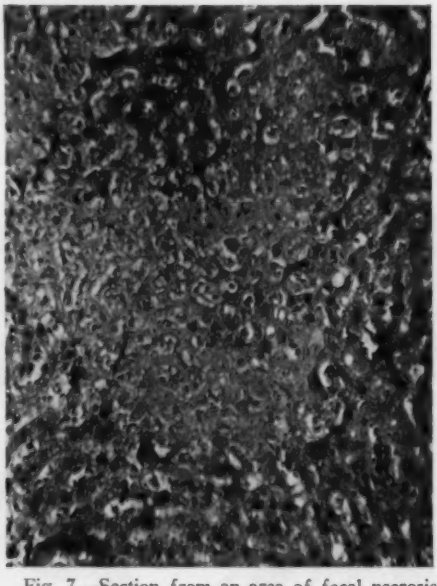

Fig. 7.—Section from an area of focal necrosis of the liver from a rat which died 48 hours after an injection of 20 mg. of sodium polyanetholsulfonate. Hematoxylin and eosin stain; reduced to 91% of mag. × 100.

terial were noted in the sinusoids. This material gave the usual staining reactions of fibrinoid.

Liver.—At early intervals following the administration of toxin and sodium polyanetholsulfonate, the liver grossly appeared to be intensely congested and enlarged. At later intervals it was sometimes possible to see scattered areas of hemorrhage or grayish-white areas of necrosis.

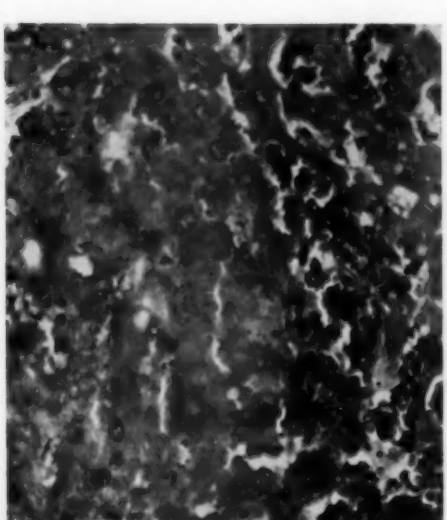

Fig. 8.—Section from a similar area of liver necrosis in an animal which died five hours after injection of toxin and sodium polyanetholsulfonate. In addition to hemorrhage, masses of conglutinated red cells and fibrinoid material are seen in the sinusoids. Hematoxylin and eosin stain; reduced to 91% of mag. × 300.

hemorrhage were observed. At later stages the liver appeared ischemic and there were large areas of necrosis (Fig. 7). Aggregates of mononuclear and polymorphonuclear cells often were present in these areas. In many cases the sinusoids appeared occluded by masses of hyaline material that were morphologically and tinctorially similar to that present in the glomerular capillaries of the kidney (Fig. 8). Occasional larger vessels showed subendothelial deposits of fibrinoid material, but no thrombi were found in these vessels.

Adrenals. — Gross adrenal hemorrhage was observed only in animals given large amounts of sodium polyanetholsulfonate alone or endotoxin in conjunction with sodium polyanetholsulfonate. It will be noted from Tables 2, 3, and 4 that meningococcal toxin administered in conjunction with sodium polyanetholsulfonate produced a significantly greater incidence of adrenal hemorrhage than did E. coli toxin and sodium polyanetholsulfonate.

Microscopically, the changes consisted of

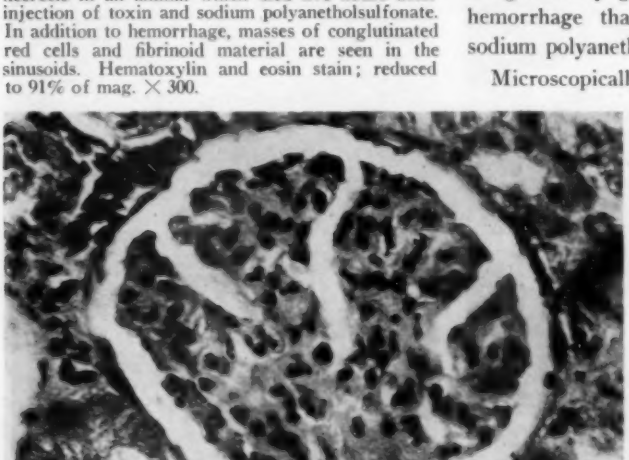

Fig. 9.—Renal glomerulus from an animal given three injections of 10 mg. of heparin sodium, in addition to toxin and sodium polyanetholsulfonate, showing minimal deposition of fibrinoid material. There is also apparently a coalescence of the capillary loops, resulting in lobulation of the glomerulus. Hematoxylin and eosin stain; × 400.

Microscopically, focal areas of hemorrhage and necrosis were observed frequently. In the animals which died four to eight hours after the administration of toxin and sodium polyanetholsulfonate marked sinusoidal congestion and areas of focal

focal areas of hemorrhage and necrosis, although occasionally the entire gland appeared hemorrhagic. In some animals hyaline fibrinoid material was present within the sinusoids of the cortex, and occasionally localized areas of ischemic necrosis were

observed, suggestive of larger vessel occlusion.

Lungs.—A very large number of animals showed extensive areas of gross hemorrhage in the lungs. Microscopically, pulmonary hemorrhage and edema were frequently found. In occasional animals thrombi of hyaline fibrinoid material were found occluding the large and small pulmonary arteries. Subendothelial deposits of fibrinoid were also occasionally observed in the pulmonary arteries.

Intestine.—Gross areas of hemorrhage in the intestinal wall were seen in several animals. Microscopically, these consisted of submucosal extravasations of blood, but no fibrinoid material was seen in any of these lesions.

Muscles.—The abdominal muscles at the injection site commonly showed gross hemorrhage. Vascular occlusion by fibrinoid material, with hemorrhage and occasional periarterial reaction of mononuclear and polymorphonuclear cells, was noted microscopically.

Testes, Brain, Pancreas.—No gross or microscopic lesions were seen in these organs.

Influence of Heparin on the Lesions

Previous reports have indicated that large amounts of heparin prevent the development of fibrinoid lesions in rabbits given two injections of endotoxin [11] or endotoxin and sodium polyanetholsulfonate.[6] In the present studies a similar experiment was carried out (Table 6) to determine the effects produced by heparin in rats given endotoxin and sodium polyanetholsulfonate. Three intraperitoneal injections of heparin were given. The first injection was given one hour prior to the administration of toxin and sodium polyanetholsulfonate; the second was given at the time of injection of toxin and sodium polyanetholsulfonate, and the third was given one hour later. Reference to Table 6 shows that in those animals given 10 mg. of heparin at each injection there was a significant decrease in the incidence of glomerular fibrinoid lesions, and in neither of the groups given heparin was gross renal cortical necrosis observed. A similar result has been observed in rabbits treated in a comparable manner.[6] It is also evident from this Table that the lethal effects of toxin and sodium polyanetholsulfonate were not prevented by heparin. In 16 of the 20 rats given heparin, however, there was considerable blood in the peritoneal cavity at autopsy, suggesting that hemorrhage may have contributed to death.

In the kidneys of nine of the animals given heparin, small amounts of fibrinoid were observed in the glomerular capillaries, and in six of these it was associated with an increased glomerular cellularity somewhat similar to that of acute proliferative glomerulonephritis (Fig. 9).

Relation of Heparin-Precipitable Fraction (HPF) to the Development of the Lesions

As a final test of the similarity of the reaction of the rat and rabbit to endotoxin and sodium polyanetholsulfonate, experi-

TABLE 6.—*Prevention of Toxin-Liquoid Lesions by Intraperitoneal Injections of Heparin**

Toxin, Cc.	Liquoid, Mg.	Heparin	No. Animals	No. Dead (24 Hr.)	Kidney Lesions	Liver Necrosis	Adrenal Hemorrhage
0.10	10	--	5	3	5	3	4
0.10	10	5 mg. × 3	10	6	6	1	2
0.10	10	10 mg. × 3	10	7	3	0	0

*Details of administration in text.

ments were performed to determine the relationship of the plasma heparin-precipitable fraction (HPF)† to the development of fibrinoid lesions. Thirty normal (control) rats were tested for the presence of this cold precipitable fraction, and it was found to be present in every animal, although in slightly varying amounts. Its cold precipitability and heat lability were similar to that of the HPF which occurs in rabbits after an intravenous injection of endotoxin.[9]

Three groups of rats were then given intraperitoneal endotoxin and sodium polyanetholsulfonate and killed at intervals of 4, 8, and 12 hours after the injection. Determinations of the HPF were carried out at these time intervals, and the results are summarized in Table 7. It will be seen that there is a steady, rather slow decline in the number of animals having this plasma precipitable fraction. This slow decline correlated well with the deposition of fibrinoid material in the renal glomerular capillaries. In the group of eight rats killed at 12 hours, for example, only one showed the presence of HPF, and in six of these microscopic examination of the kidneys showed typical hyaline fibrinoid material in the glomerular capillaries.

TABLE 7.—*Response of Plasma Heparin-Precipitable Fraction (HPF) Following Intraperitoneal Injection of Meningococcal Endotoxin and Sodium Polyanetholsulfonate (Liquoid)*

Toxin, Cc.	Liquoid, Mg.	Interval, Hr.	No. Animals	No. Positive for HPF
--	--	--	30	30
0.10	10	4	5	3
0.10	10	8	9	4
0.10	10	12	8	1

Comment

The results of the present experiments indicate that certain similarities exist in the reaction of rats and rabbits to Gram-negative bacterial endotoxin when this material is given in conjunction with the high molecular weight polymer sodium polyanetholsulfonate, and to sodium polyanethosulfonate alone when this polymer is given in large amounts. Although the rat has an apparent resistance to large amounts of endotoxin alone, the use of endotoxin combined with sodium polyanetholsulfonate markedly enhances the lethal effects and the incidence of the various morphologic changes. A similar synergistic effect has been observed in rabbits.‡

As noted previously, the generalized Shwartzman phenomenon in rabbits is characterized by the presence of bilateral renal cortical necrosis. This lesion, which appears to develop as a result of glomerular capillary occlusion by fibrinoid material, is produced in the rabbit by two properly spaced intravenous injections of Gram-negative endotoxin,§ by endotoxin in association with small amounts of sodium polyanetholsulfonate,‡ or by large amounts of sodium polyanetholsulfonate alone.[5] The present experiments have shown that an entirely similar renal lesion can be produced in the rat by the intraperitoneal administration of endotoxin and sodium polyanetholsulfonate in combination or by large amounts of sodium polyanetholsulfonate alone. With appropriate dosage levels the incidence of this renal lesion is as high in the rat as in the rabbit.

In the rat, however, the renal lesion appears to develop more slowly, and the

† References 9, 10.

‡ References 5, 6.

§ References 1, 3.

earliest detectable fibrinoid deposits are not usually seen until six to eight hours after the administration of toxin and sodium polyanetholsulfonate. One other observed difference has been the deposition of fibrinoid material in the large renal arteries of some of the rats. This lesion has not been observed in rabbits.

Certain other differences also exist. The incidence of cardiac, pulmonary, and splenic fibrinoid lesions is decidedly lower than that observed in the rabbit, but the incidence of hepatic fibrinoid and necrosis is appreciably higher. These differences may be related to the method of injection, the intraperitoneal route allowing more gradual absorption and direct access to the liver.

The prevention or modification of the renal lesion by heparin is further evidence that this reaction in the rat is similar to that in the rabbit, and that some alteration in the coagulation mechanism of the blood may be involved in the formation of fibrinoid or its precursors. The observation that glomerular cellular proliferation with minimal fibrinoid deposits was present in rats given heparin in association with toxin and sodium polyanetholsulfonate is of interest in view of Piel's statement that occasional rats given heterologous hyperimmune serum developed renal lesions similar to those of the Shwartzman phenomenon. These findings suggest that minimal or "controlled" deposition of fibrinoid in the glomerular capillaries, acting over a longer time interval, may be associated with a proliferative cellular reaction, in contrast to the capillary occlusion and necrosis produced by the "uncontrolled" deposition of large amounts of fibrinoid material seen in animals not given heparin.

The implication of the heparin-precipitable fraction in the production of the morphologic changes seen in rats in response to toxin and sodium polyanetholsulfonate also indicates the basic similarity of the reaction of both rats and rabbits. This cold precipitable material is not present in the plasma of normal rabbits, but is present after one injection of endotoxin. Following an injection of sodium polyanetholsulfonate it disappears rapidly from the plasma, its fall corresponding to the development of diffuse fibrinoid lesions.‖ In contrast to the rabbit, this material was found to be normally present in the rat, and although its rate of disappearance following injection of toxin and sodium polyanetholsulfonate is slower than that seen in the rabbit, it correlates well with the slower development of fibrinoid material in the rat. These studies suggest that this material is involved in the formation of fibrinoid in both rats and rabbits.

The similarity of the lesions in the rat and in the rabbit, the modification of the lesions by heparin, and the changes in the heparin-precipitable protein during development of the lesions suggest that similar basic mechanisms operate in the production of this reaction in both the rat and the rabbit.

Summary and Conclusions

The characteristic changes of the generalized Shwartzman phenomenon were produced in a high percentage of rats by the intraperitoneal administration of Gram-negative bacterial endotoxin in conjunction with the high molecular weight acidic polymer sodium polyanetholsulfonate (Liquoid) in appropriate dosages, or by the intraperitoneal administration of large amounts of sodium polyanetholsulfonate alone. The gross and microscopic features of the lesions were similar to those observed in rabbits, but the lesions developed more slowly and their incidence in various organs differed from that observed in rabbits. These differences appear to be due to the route of injection.

The morphologic similarity of the lesions in the rat and the rabbit, the modification of the reaction by heparin, and the changes in the plasma heparin-precipitable protein fraction during the development of the lesions suggest that similar basic mechanisms operate in the production of this reaction in both the rat and the rabbit.

‖ References 9, 10.

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Malignant Paraganglioma of the Organ of Zuckerkandl

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Emil Zuckerkandl,* in 1901, described two small structures lying on the anterior aspect of the abdominal aorta near the origin of the inferior mesenteric artery. The paired bodies are present in fetal life but undergo rapid atrophy after birth, so that they are extremely difficult to identify beyond the age of 2 years. Histologically there is a close resemblance between these so-called Zuckerkandl organs and the adrenal medulla. Both tissues also have a positive reaction with chromaffin stains, as do other scattered collections of tissues found in both humans and animals in the retroperitoneal space about the sympathetic ganglia and plexuses.

In 1903 Kohn [3] proposed the term paraganglion for extra-adrenal abdominal chromaffin tissue because of its close relation to the sympathetic nervous system, particularly in its origin. Thus, the term paraganglioma has been given to tumors of extra-adrenal chromaffin tissue. Identical neoplasms arising in the adrenal medulla have generally been called pheochromocytomas. Considerable confusion has resulted, since the terms paraganglioma, chromaffinoma, and pheochromocytoma have been used interchangeably in the literature. Both the adrenal and the extra-adrenal tumors may have physiologic function and the capacity to produce the clinical syndrome characterized by hypertension and other evidences of sympathetic overactivity.

Tumors arising from the organs of Zuckerkandl are extremely rare. Ortega,[4] in a review of the subject in 1952, recorded 14 such neoplasms and reported an additional example. The 16th case was reported by Fullerton and associates [1] in 1954. Of the tumors reported, 14 have been benign and 2 malignant, with lymph node metastases. Six of the reported cases had no clinical evidence of functional activity. The remaining 10 cases had symptoms of sympathetic overactivity, some clear-cut and others of questionable significance. Both cases with distant metastases were functioning tumors.

The following case is presented as the 17th reported tumor of the organ of Zuckerkandl. The tumor in this instance was locally invasive and metastasized to the liver. There was no clinical evidence of functional activity.

Report of a Case

A 60-year-old white male laundry worker was admitted on Nov. 28, 1954, complaining of low-back pain of five months' duration. The patient gave a history of hospitalization for "virus pneumonia" in February, 1954. He was hospitalized again in March, 1954, for intermittent claudication, tingling, and coldness of both lower extremities. Oscillometric and skin temperature studies performed with paravertebral sympathetic blocks were indicative of bilateral arteriosclerotic peripheral vascular disease. A lumbar sympathectomy was performed on each side, with symptomatic and objective improvement.

In June, 1954, the patient developed persistent pain in the left lumbosacral region, with radiation of pain into the left hip and knee. Roentgenograms of the spine revealed extensive arthritic changes. He was treated with a back brace, heat,

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From the Departments of Pathology and Surgery, Veterans Administration Hospital.

* Zuckerkandl, E., cited by Cragg.[2]

and, finally, cortisone, without relief. At the time of admission to the hospital, in November, the pain was constant, aggravated by walking or coughing, and accompanied by numbness and weakness of the left lower extremity.

Physical examination revealed the patient to be a well-developed white man, suffering from considerable discomfort in the left lower lumbar region. The blood pressure was 146/80. A smooth, nontender liver edge was palpable 6 cm. below the costal margin. There was marked limitation of motion of the lumbar spine, with tenderness and muscle spasm in the left paravertebral region. A poorly defined, firm, deeply situated mass, measuring 8×10 cm., was palpable just above the angle formed by the spine and the left iliac crest. There was weakness of the left lower extremity, with hypesthesia over the lateral aspect of the calf and absence of the knee and ankle jerks on the left. The remainder of a complete physical examination disclosed no abnormalities.

Routine laboratory studies, including serologic tests, urinalysis, complete blood counts, and determination of the erythrocyte sedimentation rate, were normal. Fasting blood sugar and blood urea nitrogen levels were normal, as were the blood levels of calcium and phosphorus. Liver-profile studies were normal except for an alkaline phosphatase of 17.1 King-Armstrong units and a sulfobromophthalein (Bromsulphalein) sodium retention of 19% in 45 minutes.

Roentgenograms of the lumbar spine revealed a destructive process involving the pedicle, the pars interarticularis, and two processes of the fourth lumbar vertebra. There was nonvisualization of the psoas muscle shadow on the left, although the muscle on the right was readily visible. There was also a suggestion of increased soft-tissue density to the left of the third and fourth lumbar vertebrae. Extensive osteoarthritic involvement of the spine was evident, as well as calcification of the abdominal aorta. An intravenous urogram was normal except for rather poor visualization of the left renal shadow.

On Dec. 17, an attempt was made to obtain a biopsy specimen of the left lumbar mass by a posterior approach. A vascular, yellow-gray tumor was discovered lying within the quadratus lumborum muscle and invading the spine. Upon incision into the tumor profuse hemorrhage was encountered, necessitating packing of the wound and administration of 1000 cc. of whole blood. The tissue removed at operation was reported by the pathology department as adenocarcinoma, possibly metastatic from the kidney or adrenal gland.

Following healing of the operative wound, the patient was discharged from the hospital at the request of his relatives, so that he might receive a drug claimed to be of value in the treatment of cancer (Krebiozen). This substance was administered at regular intervals by his local physician until the final hospital admission.

The patient was readmitted on June 8, 1955, in a moribund condition. There was marked cachexia, with wasting of the extremities. The abdomen was distended and tympanitic; there was a large sacral decubitus. Death occurred eight hours after admission.

Fig. 1.—Gross appearance at autopsy. The tumor is immediately adjacent to the bifurcation of the aorta and invading the left psoas muscle. *A*, aortic bifurcation; *B*, inferior mesenteric artery; *C*, tumor.

Autopsy Findings

The body was that of a poorly nourished man with moderate edema of the lower extremities and diffuse muscle wasting. There were healed scars over the flanks and the left lumbar region. The significant gross findings were confined to the abdomen. Located retroperitoneally, in the left paravertebral region, immediately adjacent to the bifurcation of the aorta lay a bulging, lobulated fusiform mass, partially covered by, and invading, the psoas muscle (Fig. 1). The mass measured 18×6 cm. and encompassed many of the lumbar plexus nerves. It extended directly to invade the adjacent vertebral bodies, the superior part of the posterior iliac crest, and the extradural tissues about the lower spinal cord and the cauda equina, and transgressed the vertebral arch to penetrate the long spinal muscles. There was no invasion of the spinal cord itself. On section the tumor showed pseudoencapsulation with streaks of fibrous tissue.

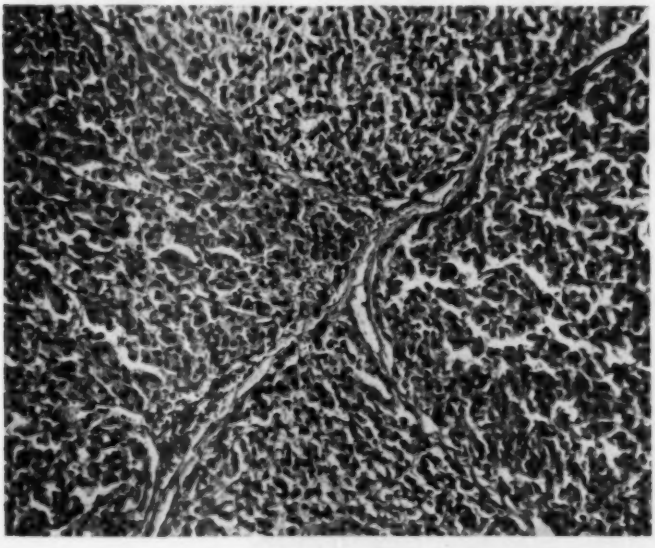

Fig. 2.—Medium-power view of primary tumor. Note syncytial pattern with sparse stroma. Reduced to 64% of mag. × 100.

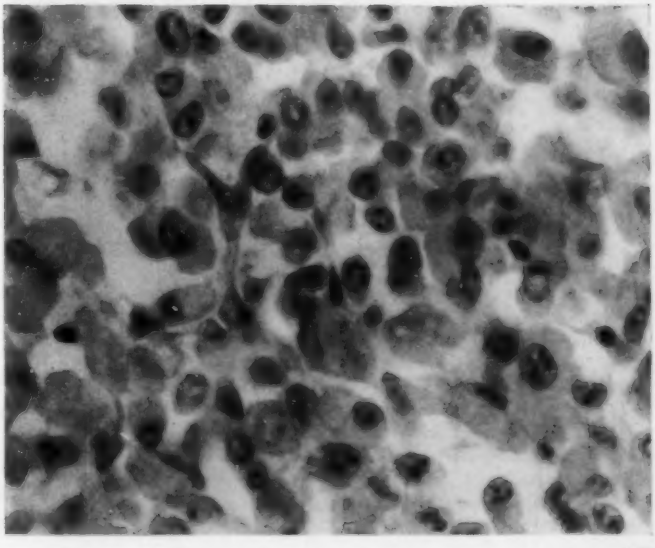

Fig. 3.—High-power view of primary tumor. Large epithelial-like cells contain rounded and pleomorphic nuclei. Reduced to 64% of mag. × 550.

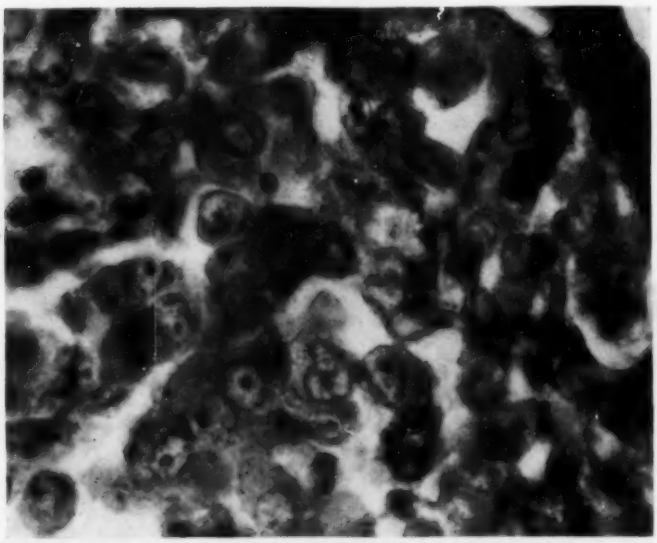

Fig. 4.—Gomori chromaffin stain, demonstrating cytoplasmic granules with positive chromaffin reaction. Reduced to 64% of mag. × 650.

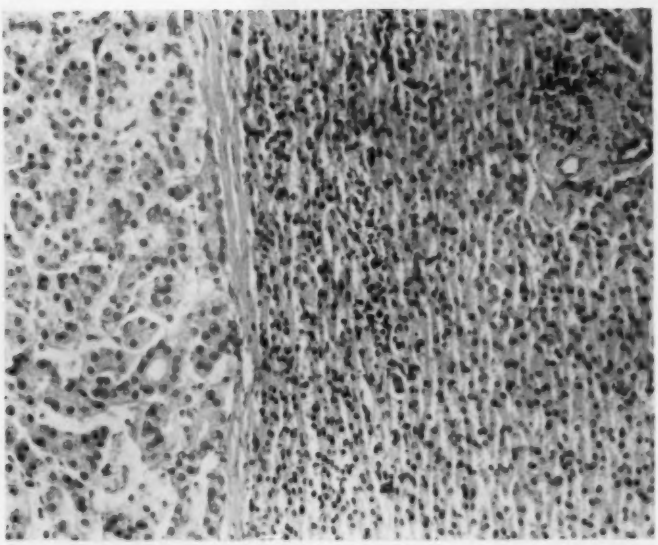

Fig. 5.—Metastatic deposit in liver, with compression of normal liver tissue. Reduced to 64% of mag. × 100.

It was soft and dark-brown, with many honeycombed cystic areas of degeneration present. Much of the tumor was necrotic and friable.

The liver was enlarged and weighed 3950 gm. On section the organ was seen to be literally replaced by metastatic tumor, the nodules of which measured from 1 mm. to 8 cm. in size. These nodules were generally brown, but some were yellow or green.

Other findings included numerous, somewhat enlarged, mesenteric lymph nodes, slightly granular kidneys, hemorrhagic cystitis with calculi, pulmonary edema and congestion, healed fibrous pleuritis, and bilateral hydrothorax.

Microscopic Examination

The primary tumor was composed of solid sheets of large polygonal and elongated epithelial-like cells, arranged in groups, separated by bands of vascularized connective tissue. Sometimes the tumor was

formed of a syncytium of cells; at other times the cells were in poorly arranged clumps and groups. The cells had an abundant eosinophilic cytoplasm with poorly defined cell margins and rounded or pleomorphic dark nuclei. The chromatin generally appeared stippled, and nucleoli were often present. Mitotic figures were rarely seen (Figs. 2 and 3). The stroma was edematous and generally poorly cellular, but in some areas hyalinized fibrous tissue was seen, containing focal areas of calcification, lymphocytic infiltration, and hemosiderin deposition. The adjacent nerve and striated muscle fibers showed compression atrophy, invasion, degenerative changes, and interstitial edema. Silver stains [5] revealed the presence of delicate strands of connective tissue between groups of tumor cells, and even between some individual cells, and disclosed many small additional vascular channels not previously observed. Gomori's chromaffin tissue stain [2] on formalin-fixed tissue revealed faint, but definite, cytoplasmic granules, having a pink to red coloration, best seen under the oil immersion lens (Fig. 4).

The metastatic lesions replacing the liver parenchyma appeared virtually identical with the parent tumor except that the cytoplasm of some cells appeared basophilic (Fig. 5). A rare gland-like arrangement of the cells was also observed. Sections of the lumbar vertebrae disclosed sheets of tumor cells filling the narrow spaces with destruction of many trabeculae. Small tumor emboli were seen in pulmonary capillaries but none in the lung parenchyma. The mesenteric lymph nodes showed reactive hyperplasia and chronic inflammation. The adrenal glands were free of tumor.

Comment

Microscopic sections of the tumor were examined by Dr. Frank W. Foote at Memorial Hospital, New York, who agreed with the diagnosis of paraganglioma. The site of the neoplasm, just lateral to the bifurcation of the abdominal aorta, suggests that it originated in the organ of Zuckerkandl. This tumor is the third malignant paraganglioma reported to have arisen in this location.

Assay for epinephrine was not performed on the tissue obtained at operation or at autopsy. At no time during the hospitalization of the patient was hypertension or other evidence of sympathetic overactivity observed. Although the two malignant paragangliomas of the organ of Zuckerkandl previously reported were both functioning tumors, the great majority of malignant paragangliomas arising in other sites, including the adrenal medulla, have shown no evidence of physiological activity.

Dean Altman assisted in the preparation of illustrations and photomicrographs.

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News and Comment

PERSONAL NEWS

Dr. S. M. Rabson Joins Coroner's Staff of Los Angeles.—Dr. S. M. Rabson, director of the department of pathology, St. Joseph Hospital, Fort Wayne, Ind., has joined the Coroner's staff of Los Angeles.

Dr. Stuart W. Lippincott Appointed Pathologist to Brookhaven National Laboratory.—Dr. Stuart W. Lippincott has been given a senior appointment in the division of experimental pathology and has been appointed pathologist to Brookhaven National Laboratory, at Upton, N. Y.

Dr. Max B. Lurie Awarded Trudeau Medal.—Dr. Max B. Lurie, professor of experimental pathology at the Henry Phipps Institute of the University of Pennsylvania, was awarded the 1956 Trudeau Medal by the National Tuberculosis Association in recognition of his studies on problems of native and acquired resistance to tuberculosis.

Dr. Philip C. Pratt goes to Ohio Tuberculosis Hospital.—Dr. Philip C. Pratt, former assistant director and research pathologist at the Saranac Laboratory in New York, has become chief of laboratories at Ohio Tuberculosis Hospital, in Columbus.

Col. Hugh R. Gilmore Jr. Retires as Curator of Medical Museum.—Col. Hugh R. Gilmore Jr. has retired from the U. S. Army as Curator of the Medical Museum of the Armed Forces Institutes of Pathology. Colonel Gilmore has held this post since 1953 and has been unusually active in getting the affairs of the Medical Museum organized in the new Armed Forces Institutes. He was recently cited by Maj. Gen. Silas B. Hays, Surgeon General, U. S. Army, for 30 years of superior and devoted service to the Army Medical Service.

GENERAL NEWS

Jean Redman Oliver Lecture.—The annual Jean Redman Oliver Lecture was presented this year by Dr. Homer W. Smith, of New York City, who discussed "The Development of Modern Renal Physiology."

SOCIETY NEWS

American Association for the Study of Neoplastic Diseases.—At the annual meeting of the Association, held at the Drake Hotel in Chicago, June 30-July 2, Dr. Elizabeth A. McGrew, of the department of pathology of the University of Illinois, discussed "Exfoliative Cytology"; Dr. Paul E. Steiner, of the University of Chicago, discussed "Some Observations on the Etiology of Lung Cancer," and Dr. Philippe Shubik, of the Chicago Medical College, talked on the subject "Carcinogenesis as a Problem in Chronic Toxicity Testing." A microscopic session was also conducted.

ANNOUNCEMENTS

Increased Charge for Stain Certification.—The Board of Trustees of the Biological Stain Commission has found it necessary to increase the price of Certification Labels by 5¢, effective Sept. 1, 1956. This will result in a change from the current charge of 15¢ to a new charge of 20¢ per bottle to the purchasers of certified stains. This price increase has been necessitated by increasing costs in the operation of the Commission and is the first such increase in almost 20 years.

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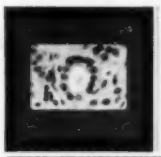

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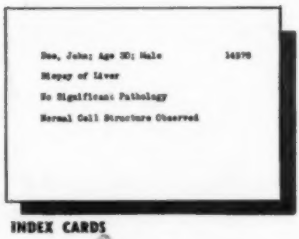

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